On the Alcohol-Soluble Proteins of Naked Barley.

By

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I. Introduction.

Many studies on the protein of barley, carried out up to this time, as far as the authors are aware, have been solely limited to the common barley.

No literature could be found regarding the naked barley, which is a no less important kind of crop in our daily life.

An alcohol-soluble protein in the seed of barley was described as early as 1805 by Einhof.[1]

In 1848, Mulder[2] obtained a plant-gelatin, precipitated by cooling the hot alcohol extract of barley meal and determined further its elemental composition.

In 1861, Bibra[3] identified Mulder's plant-gelatin as the barley protein.

Kreusler made an investigation of the barley protein, the results of which were published by Ritthausen[4] in 1872. He extracted the barley flour with hot 75 per cent alcohol and by fractional precipitation with acetic acid isolated the three proteins : gluten-casein, gluten-fibrin and mucedin. Through analysis he pointed out the differences of the elemental composition among them.

Osborne[5] prepared many alcohol-soluble proteins by various original methods, and their elemental compositions were also analyzed.

According to him, no differences were found in those proteins and he concluded that alcohol-soluble protein of barley was a homogeneous substance, not a mixture as had been stated by previous scholars.

He proposed a name "Hordein" for the alcohol-soluble protein of barley. But, for the identification of the proteins, elemental analysis was usually employed at that time. It appears, therefore, that the differences were not clearly defined.

Later, Osborne and Clapp (1907)[6], Kleinschmitt (1907)[7], Johns

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and Finks (1919)[8] tried hydrolysis of hordein and determined the proportion of amino acids or amino nitrogen.

Thus it was made clear that gliadin, which resembles hordein in elemental composition, differs distinctly from hordein in the proportion of amino acids which are yielded from hydrolysis. By the same studies, the chemical constitution of hordein was greatly elucidated.

Recently, with the progress of colloidal chemistry, the delicate character of the proteins has been studied in our biochemical laboratory. Tadokoro and Nakamura[9] having studied the oryzenin of rice, found clear differences between common and glutinous rice oryzenins.

The present authors made a preparation of alcohol-soluble protein of naked barley with three different kinds of proteins and studied on some characters of these proteins from the physico-chemical and pure chemical view points. The present paper embodies the result of these studies.

II. General Analysis of Naked Barley and Quantitative Analysis of Various Proteins.

The naked barley used in this analysis, was of the produce of Hokkaido. The name of the cultivated variety thus used is "*marumi*".

The grain was ground to flour by a stone mill, and its chemical composition analyzed: moisture 12.90%, ash 1.83%, crude protein 13.66%, crude fat 2.76%, crude fiber 2.47%, soluble non-protein substance 66.38%.

For the quantitative determination of the various proteins, one gram of the flour was weighed into each flask and well washed by water several times with frequent shakings. The residue was washed next just as above with 10% NaCl solution, its residue with 70% alcohol, at last with 0.2% NaOH solution. Then the nitrogen in each residue was determined by Kjeldahl's method.

Total nitrogen of the flour was also determined and the quantity of nitrogen in the various forms of protein was obtained by calculation. The following data were obtained by multiplication by 6.25.

Crude protein	13.6%
Water soluble	1.0%
10% NaCl solution soluble	2.1%
70% alcohol solution soluble	3.3%

0.2% NaOH solution soluble	4.0%
Insoluble	3.2%

III. The Preparation of the Alcohol-Soluble Proteins from the Naked Barley and their Purification.

For the preparation of the alcohol-soluble protein from the naked barley, Osborne's method[10] was somewhat modified.

Samples were of the same variety used in the general analysis. They were taken from the yields of 1924 and 1925.

The proteins were prepared in September 1925 from the former yields and in November of the same year from the latter.

1. Four and eight tenths kilograms of the flour of 1924 yield were successively extracted with 70% alcohol; 1.2 kg. out of 4.8 kg. of the flour was first extracted three times with 70% alcohol, then the extract solution from the above was added to the next 1.2 kg. portion of the fresh flour with complementing alcohol to make a volume to twice that of the flour. Being frequently shaken, it was left to stand for one day at room temperature. Then it was filtered. The filtered solution was clear yellowish red in color. The filtrate was added to the next fresh flour of 1.2 kg. weight and extraction was proceeded with again as before.

The combined alcohol extracts were then concentrated to small volume under diminished pressure at 50°C., until the slight white turbidity of the protein appeared, then transferred to beaker which was then placed in an ice box over-night.

A jelly-like substance settled to the bottom of the beaker.

The upper solution was then decanted, the precipitated substance dissolved again in alcohol and poured into 8–10 volumes of cold distilled water at 0–5°C. with constant agitation. A coagulated substance was formed instantly.

This coagulum was dissolved again in a small quantity of alcohol and manipulated three times in the same manner as above to remove the water soluble matters.

The yellowish white coagulum was dissolved in a small quantity of alcohol, poured it into 7–8 volumes of absolute alcohol in a narrow stream from the beaker. A dehydrated hard coagulum was formed and the alcohol solution changed to turbid white. After the filtration, the coagulum was placed again in absolute alcohol for

5–6 hours, transferred next to the acetone until dehydrated, freed from lipoid and washed with ether at last.

The substance was then pulverized to a white powder while wet, the ether removed and next milled in a porcelain dish. This substance was named A. The milky turbid solution obtained from the above operation was condensed to small volume under a diminished pressure at 40°C. When alcoholic content of the solution was lowered to about 70% by addition of a little water, it became clear yellowish in color. When this solution was poured into absolute alcohol it changed to turbid white again, but no coagulum was produced. Therefore the solution was poured into cold distilled water as before, whereupon a coagulated substance formed. After the same treatment of dehydration and purification as above a light yellowish white powder was obtained. This substance was named B.

2. Four and eight tenths kilograms of the 1925 yield were taken and two kinds of proteins were prepared after the same methed as above. They were named C and D respectively. The substance C corresponding to A was somewhat different from it in the nature of the coagulum. This substance was not so elastic in cold water as A. In absolute alcohol, it was not coagulated so hard as A, but a soft silk-like substance resulted.

Another substance D corresponding to B was very like B in the nature of the coagulum, the quantity was less than that of B.

The yield of the proteins from the flour, their moisture and ash contents resulted as follows:—

Prepared sub.	Yields (from 4.8 kg.)	Ash	Moisture
A	47 gm.	0.05%	4.61%
B	8	0.10	4.70
C	20	0.20	8.91
D	0.5	—	—

IV. Physico-chemical and Pure Chemical Properties of Proteins A, B, C and D.

1. The Color Reactions of Protein.

Of the newly separated substances A, B, C and D, various color reactions of protein were tested.

TABLE I.

Color Reactions of the Alcohol-soluble Proteins obtained from the Naked Barley.

Color Reaction	A	B	C	D
Biuret reaction.	Violet.	Beautiful violet.	Violet.	Beautiful violet.
Millon's reaction.	White precipitate occurs, when heated it turns reddish brown.	White precipitate occurs, when heated it turns reddish brown.	White precipitate occurs, when heated it turns reddish brown.	White-precipitate occurs, when heated it turns reddish brown.
Tryptphane reaction.	Violet ring occurs.	Brownish violet ring formed.	Violet ring occurs.	Brownish violet ring formed.
Nitric acid reaction.	White precipitate does not occur. yellow.	White precipitate does not occur. yellow.	White precipitate does not occur. yellow.	White precipitate does not occur. yellow.
Hydrochloric acid reaction.	Dark red.	Dark red (Precipitate occurs)	Beautiful reddish violet.	Dark red (Precipitate occurs.)
Conc. sulphuric acid reaction.	Red–dark red.	Red–dark brownish yellow.	Beautiful reddish violet–deeply reddish violet.	Red - dark brownish yellow.

B and D gave a similar reaction.

2. The Behavior of Proteins A, B, C and D toward Alcohol, NaOH, HCl, NaCl Solutions and Distilled Water.

The behavior of proteins A, B, C and D toward alcohol, NaOH, HCl, NaCl solutions and distilled water, was next observed.

TABLE II

The Behavior of Proteins A, B, C and D toward Alcohol, NaOH, HCl, NaCl Solutions and Distilled Water.

Reagents	A	B	C	D
Distilled water.	Insoluble.	Insoluble.	Insoluble.	Insoluble.

	A	B	C	D
70% cold alcohol.	Dissolved.	Dissolved with difficulty.	Easily dissolved.	Dissolved with Difficulty.
70% warm alcohol.	Easily dissolved.	Dissolved.	Rapidly dissolved.	Dissolved.
Absolute alcohol.	Coagulated firmly.	Made an emulsion.	Coagulated softly.	Made an emulsion.
N100/ NaOH solution.	Dissolved (yellow).	Dissolved with difficulty (brownish yellow).	Easily dissolved (yellow).	Dissolved with difficulty (brownish yellow).
N/100 HCl solution.	,,	,,	,,	,,
NaCl, (into alcohol solution of Proteins)	Precipitate.	Precipitate.	Precipitate-	Precipitate.

B and D in these tests behaved in the same way.

3. White Turbid Phenomena of Proteins in Relation to the Concentration and the Temperature of Alcohol.

A white turbidity occurs when the alcohol solution of protein was warmed or cooled and diluted or concentrated with distilled water or alcohol. These phenomena were observed in relation to the concentration and the temperature of alcohol and the differences in the behavior among the proteins A, B, C and D were found.

One-tenth gram (this amount calculated as anhydrous substance) of each protein, A, B, C and D was taken into the test tubes and dissolved completely in 10 cc. of 70% alcohol.

To the above solution a certain amount of distilled water was added from a burette, with constant agitation by the thermometer inserted in the test tube. It was then cooled in ice water or warmed in the water bath until the white turbidity just began to appear at which point the thermometer was read. Tracing the critical points at which the white turbidity occurs, the curves shown below were obtained.

TABLE III.

White Turbidity of Protein in Relation to the Concentration and Temperature of Alcohol.

Titration No. of water (cc.)	The temperature at which turbidity occurs (°C.)						
	A		B		C		D
0	14	14	7	7	14	14	7
1	11.5	11.5	7	7	11.5	12	7
2	13	13	8	9	13	13	8
3	16	15	12	15	15	16	12
4	19	18	16	17	18	19	17
5	21	21	21	22	21	22	21
6	26	24	25	26	25	25	25
7	27	27	30	30	29	27	30
8	30	28	32	34	32	21	31
9	32	32	36	38	35	33	36
10	35	34	41	43	38	38	42
11	37	37	44	47	—	40	45
12	—	39	47	—	—	42	—
13	—	41	51	—	—	—	—
14	44	44	55	—	—	—	—
15	—	—	61	61	54	50	61
16	50	—	64	—	—	—	—
17	—	50	—	—	56	—	—
18	58.5	—	74	75	—	59	—
19	—	—	—	—	—	—	—
20	61.5	60	85	85	63	63	85
21	—	—	—	—	—	—	—
22	—	—	93	(Boil) 93	—	—	(Boil) 93
23	—	70	—	—	66	—	—
24	71.5	—	—	—	—	—	—
25	—	—	—	—	—	70	—
26	—	75	—	—	—	—	—
27	—	80	—	—	—	—	—
28	—	—	—	—	—	73	—
29	—	88	—	—	—	—	—
30	—	—	—	—	78	76	—
31	—	—	—	—	—	—	—
32	94	(Boil) 93	—	—	—	—	—

Titration No. of water (cc.)	The temperature at which turbidity occurs (°C.)						
	A		B		D		
33	—	—	—	—	82	—	—
35	—	—	—	—	—	—	—
36	—	—	—	—	86	82 (Boil)	—
40	—	—	—	—	—	91	—

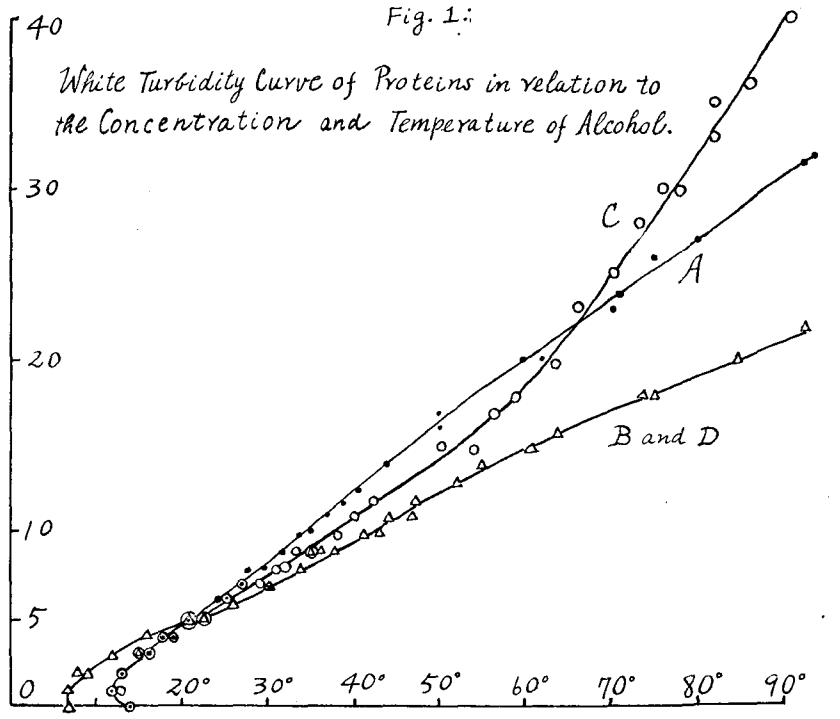


Fig. 1. shows, when 5 cc. of distilled water, for example, were added, the alcohol solution of each of the proteins A, B, C and D was turbid if the temperature at the time were 5°C., but if the temperature were raised to 80°C., all the solutions became clear.

If the temperature were lowered to 63°C., B solution gradually became turbid while A and C solutions remained clear.

If the temperature were lowered further to 53°C., B became more turbid and C gradually turbid, while A still remained clear.

But A became turbid if the temperature were lowered to 48°C.

Such a white turbid phenomenon differs with the individual protein as is seen in Fig. 1., excepting at the point represented by 21°C. in the ordinate and 5 cc. (it's concentration corresponding to 47% alcohol) in the abscissa.

In another experiment, we tried what percentage of alcohol would be most favorable to extract the maximum quantity of protein at room temperature (about 20°C.).

To 1 gm. each of the naked barley flour 50 cc. of different per cent of alcohol were added as seen in the following table, left one day, shaking frequently and filtered. Thereupon the nitrogen in the residue was determined, and soluble nitrogen was then calculated from it.

Percentage of soluble nitrogen to the total nitrogen of the flour was as follows:—

TABLE IV.

The Solubility of Protein in the Various Percentages of Alcohol Solution at room temperature (about 20°C.).

Percentage of alcohol	Percentage of nitrogen soluble in the alcohole solutions to the total nitrogen of the flour
0 (water)	6.9
10	12.3
20	15.4
30	20.0
40	23.1
50	27.7
60	27.7
70	27.7
80	17.0
90	14.6

As can be seen in the above table the solubility of protein is at the maximum in 50% alcohol at room temperature and the solu-

bility in the strong alcohols is higher than that of low per cent alcohols. Comparing this results to those facts, stated in Fig. 1. on the preceding page, we can find the following facts. When the temperature is at room temperature (about 20°C,) and at the same time, the titration number of water is 5 cc. which corresponds to 47% in alcohol concentration, the turbidity just appears, but it becomes greater if the titration number is more than 5 cc. (less than 47%). On the other hand the turbidity is decreased at less than 5 cc., (more than 47%) with maximum solubility of protein at 50–70% of alcoholic concentration as shown in Table IV.

Furthermore, that the solubility of the protein is higher in a strong alcohol solution seems to us to be probable, owing to the fact which we have seen above in heading 2 of section IV where it is shown that a protein such as B exists as a milky solution, which does not become a coagulated mass, even in the absolute alcohol.

In this test, proteins B and D were also the same in their behavior.

4. The Variation of the Surface Tension resulting from the Titration of HCl into the NaOH Solutions of the Proteins.

Proteins A, B and C were completely dissolved in NaOH solution. The variation of the surface tension with the titration of HCl into these protein solutions was observed. (D was not used in these experiments).

One-tenth gram of protein (calculated as anhydrous) added to 10 cc. of N/50 NaOH solution, was left over night until completely dissolved. One cc. of it was added to 7 cc. of pure water (pH 7.0) and the surface tension was measured by du Noüy's apparatus at each titration by N/100 HCl.

The results are shown in Table V.

The numbers in the table are those of the degree on the apparatus which is graduated to be proportional to the surface tension.

TABLE V.

The Variation of the Surface Tension upon the Titration of HCl into the NaOH Solution of the Protein.

Titration cc. of N/100 HCl.	Surface Tension degree		
	A	B	C
1.6	77.5	72.0	82.5
1.65	—	70.0	—
1.70	76.5	69.5	78.0
1.72	—	—	76.0
1.74	—	—	76.5
1.75	76.5	69.0	—
1.76	—	—	76.5
1.78	—	—	76.5
1.80	75.0	66.5	76.0
1.82	—	—	76.5
1.84	75.5	—	77.6
1.85	—	68.5	—
1.86	—	—	77.0
1.88	76.0	—	78.0
1.90	75.5	69.0	77.5
1.92	75.0	—	77.0
1.94	75.0	67.0	77.0
1.95	—	—	—
1.96	74.5	67.5	76.5
1.98	75.0	69.0	78.0
2.00	76.0	—	77.0
2.02	—	69.0	76.7
2.04	75.0	69.0	76.0
2.05	—	—	—
2.06	75.0	69.0	76.0
2.08	74.8	69.0	77.5
2.10	74.0	70.0	77.0
2.12	74.5	—	76.0
2.14	74.8	70.0	76.0
2.16	74.9	70.3	76.0
2.18	75.6	70.0	76.0
2.20	—	70.0	76.0
2.22	—	70.0	76.5
2.24	74.9	70.0	78.0

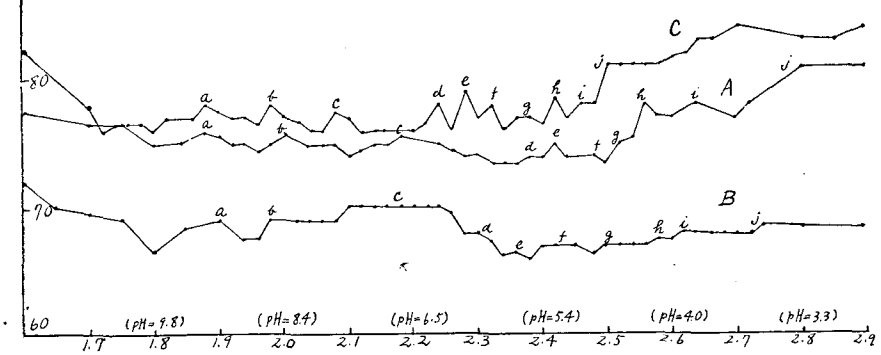
Titration cc. of N/100 HCl.	Surface Tension degree		
	A	B	C
2.26	74.5	69.5	76.0
2.28	74.0	68.0	79.0
2.30	73.9	68.0	77.0
2.32	73.0	67.5	78.0
2.34	73.5	66.7	76.0
2.36	73.5	66.5	77.0
2.38	74.0	66.0	77.0
2.40	74.0	64.0	76.5
2.42	75.1	67.0	78·5
2.44	74.0	—	77.0
2.45	—	66.8	—
2.46	—	—	78.0
2.48	74.0	66.5	78.0
2.50	73.5	67.5	81.0
2.52	75.0	67.0	81.0
2.54	75.2	67.0	81.0
2.56	78.0	67.0	81.0
2.58	77.0	67.5	81.0
2.60	77.0	67.5	81.5
2.62	—	68.0	82.0
2.64	78.0	68.0	83.0
2.66	—	68.0	83.0
2.68	—	68.0	—
2.70	77.0	68.0	84.0
2.72	78.0	68.0	—
2.74	—	68.5	—
2.80	81.0	68.5	83.0
2.85	—	—	83.0
2.90	81.0	68.5	84.0
3.00	—	68.5	83.0
3.10	—	—	86.0
3.20	—	—	84.5

As is seen from the above table and following figure, the surface tensions of A and C are somewhat similar to each other but

they are higher than B.

Fig. 2.

The Variation of the Surface Tension by the Titration of HCl into the NaOH Solutions of the Proteins.



The variation of the curves becomes gradually less sharp in the order C, A, B. If these curves be carefully observed, it will be seen that the individual curves are mutually analogous that is, the points indicated on the curves by small letters are mutually analogous points. But the places of these points for the abscissa as show in the curves of A and B are alike while they differ greatly from those of C.

5. Elemental Composition of Proteins A, B and C.

The elemental composition of Proteins A, B and C were analyzed with results as follows.

Nitrogen was measured by Kjeldahl's method, sulphur by Denis-Benedict's method, carbon and hydrogen by the usual method.

Oxygen was calculated by subtracting the sum of the per cent of the above elements from 100.

For comparison the results determined by other authors are mentioned in the following table:—

TABLE VI.

Elemental Composition of Proteins.

Protein			C	H	N	S	O
		A	55.97%	6.64%	16.36%	0.33%	20.70%
		B	54.72	6.50	15.18	0.63	22.97
		C	56.16	6.65	17.15	0.19	19.85
Plant-gelatin (Mulder)			54.85	7.05	15.71	0.60	21.79
,, (Bibra)			—	—	15.5-15.6	—	—
(Ritthausen)	Gluten-casein		53.55	7.15	16.63	—	—
	Gluten-fibrin	from meal	55.23	7.24	15.49	22.04	
		from flour	54.55	7.27	15.70	22.48	
	Mucedin	from meal	53.19	6.65	16.14	24.02	
		from flour	53.97	7.03	16.98	0.68	21.34
Hordein (Osborne)			54.29	6.80	17.21	0.83	20.87

It was found that when the proteins are ranged in the order C, A, B, the per cent of carbon, hydrogen and nitrogen gradually decreases, while that of sulphur and oxygen increases.

In comparing these results with those of elemental analysis made by other authors, A is like gluten-fibrin, while B is like plant-gelatin or mucedin, or somewhat like the hordein of Osborne.

6. The Analysis of the Various Forms of Nitrogen from the Hydrolysis of the Proteins.

The proteins were analyzed by Van Slyke's method[11] to discover the proportion of various forms of nitrogen.

With 20 per cent hydrochloric acid 3 gm. of protein were treated for 30 hours on the sand bath. After complete hydrolysis, and a greater part of the hydrochloric acid had been evaporated off, 100 cc. of strong alcohol and 10 per cent of calcium hydroxide solution were added. The amide nitrogen was, then, determined by distillation.

After filtration, humine nitrogen of the residue was determined. The filtrate was made neutral by hydrochloric acid, and condensed to small volume from which diamino acid was precipitated as phosphotungstate.

After standing for 48 hours, the precipitate was filtered and the diamino nitrogen was determined by Kjeldahl's method, decomposing the precipitate by conc. sulfuric acid.

Monoamino nitrogen was calculated by subtracting the sum of amide, humine and diamino nitrogens from the total nitrogen.

The results are to be seen in Table VII.

The proportion of various forms of nitrogen to the total Nitrogen is shown also in Table VIII.

The figures in the table were calculated on the basis of ash and moisture free, and the solubility correction of phosphotungstate was not made.

For contrast, the analytical result obtained by Osborne and Harris (1903)[12] is cited.

TABLE VII.

The Proportion of the Various Forms of Nitrogen.

	Total N	Amide N	Numine N	Diamino N	Mono-amino N
A	16.36%	3.03%	0.22%	2.70%	10.41%
B	15.18	2.00	0.22	2.24	10.72
C	17.15	4.00	0.31	3.01	9.83
Hordein (Osborne & Harris)	17.21	4.01	0.23	0.77	12.20

TABLE VIII.

The Ratio of Various Forms of Nitrogen to the Total Nitrogen.

	Total N	Amide N	Humine N	Diamino N	Mono-amino N
A	100%	18.52%	1.34%	16.50%	63.64%
B	,,	13.17	1.45	14.75	70.64
C	,,	23.32	1.81	17.56	57.31
Hordein (Osborne & Harris)	,,	23.00	1.70	7.69	67.61

From the above data, amide nitrogen and diamino nitrogen gradually decrease in the order C, A, B, but on the contrary monoamino nitrogen gradually becomes higher. We also note in Table VIII, that the degree of decrease of amide nitrogen greater in comparison with that of diamino nitrogen.

7. The Measurement of Cystine in the Hydrolytic Products of Proteins.

Since Van Slyke[13] in 1911 found the method of measuring cystine as phosphotungstate, this method has been hitherto generally used by many investigators.

But recently, in 1922, Hoffmann and Gortner[14] indicated that when the cystine was boiled with acid, a part of the cystine was changed to an isomer, the tungstate of which was easily soluble.

In the same year, Folin and Looney[15] proposed the colorimetric method with uric acid reagent. In 1925, Okuda[16], observing similar color reactions with uric acid reagent shown by the compounds other than cystine existing in the protein molecule, whereby the method of Folin and Looney leads to faulty results, proposed his iodine method.

According to his method, even if the quantity of cystine be very small, it may be measured with a great degree of accuracy notwithstanding the existence of the other amino acids. Then, the present authors used Okuda's method.

With 5 cc. of conc. HCl (Sp. gr.=1.19) 1 gm. of protein (calculated as anhydrous) was first boiled with the reflex condenser on the water bath and then on the sand bath for 20 hours.

After hydrolysis, the greater part of the HCl was evaporated off under diminished pressure and decolorized. The cystine was then reduced to cysteine with zinc powder and the total volume was made up to 100 cc. the concentration of HCl being made exactly 2 per cent. From the above solution 20 cc. were taken, mixed with 5 cc. each of 5 per cent potassium iodide and 4 per cent hydrochloric acid solution, then titrated with 1/300 mol potassium iodate. The content of cystine was calculated making correction for the number of potassium iodate titration.

The results are as follows. The other results obtained by Johns and Finks[17], and Lüers[18] for hordein are cited for comparison.

TABLE IX.

	Cystine	Cystine N	Cystine N (expressed in per cent of total N.)
A	0.8734%	0.1018%	0.64%
B	1.4792	0.1725	1.07
C	0.2294	0.0266	0.15
Hordein (Johns & Finks)	—	—	1.18
,, (Lüers)	—	—	1.58

A clear difference was found between A, B and C but it was small, as seen in the above authors' figures.

It will be worth while to study the ratio of cystine sulphur to total sulphur.[19]

It has been demonstrated that many proteins yield cystine on hydrolysis, and the greater part, if not all, of their sulphur, is considered to be cystine sulphur.

TABLE X.

The Ratio of Cystine Sulphur to the Total Sulphur.

	Total S	Cystine S	Cystine S/Total S
A	0.3284%	0.2333%	0.710 (2/3)
B	0.6327	0.3951	0.625 (2/3)
C	0.1934	0.0613	0.317 (1/3)

V. On the Differences of Proteins Separated from the Naked Barley and Comparison with Hordein Obtained by Osborne.

The authors extracted the alcohol soluble proteins from the new and old naked barley by means of 70 per cent alcohol, and the above mentioned proteins A, B, C and D were separated.

But, the proteins B and D were considered to be the same substance from the coincidence of their color reaction, solubility for solvents and behavior of white turbidity in relation to the concentration and temperature of alcohol.

But the proteins A, B and C were considered to be different from one another.

The differences in the nature of these proteins taken up in our special investigation are as follows:—

(1) The differences in the color reaction of the proteins.
(2) ,, ,, in the behavior in their relation to the solvents.
(3) ,, ,, in the white turbidity curves.
(4) ,, ,, in the surface tension curves.
(5) ,, ,, in the elemental composition.
(6) ,, ,, in the hydrolytic products by HCL.
(7) ,, ,, in the ratio of cystine sulphur to the total sulphur.

Differences in (2), (3) and (4) are refered the colloidal nature of the protein, while those in (1), (5), (6) and (7) to the chemical composition.

Now, the results of the above experiments, comparing the proteins obtained from the naked barley by the authors with those from the common barley by Osborne, may be summarized in the following table:—

TABLE XI.

Summary Comparison of the Alcohol-Soluble Proteins from Naked and Common Barley.

		Hordein (by Osborne)	Alcohol Soluble Proteins of Naked Barley (by Authors)		
			C	A	B
Color Reaction to conc. H_2SO_4		red	reddish violet	red	red
Elemental Composition	Carbon	54.29%	56.16%	55.97%	54.72%
	Hydrogen	6.80	6.65	6.64	6.50
	Nitrogen	17.15	17.15	16.36	15.18
	Sulphur	0.83	0.19	0.33	0.63
	Oxygen	20.87	19.85	20.70	22.97
Hydrolysis by HCl	Total N	17.21%	17.15%	16.36%	15.18%
	Amide N	4.10	4.00	3.03	2.00
	Humine N	0.23	0.31	0.22	0.22
	Diamino N	0.77	3.01	2.70	2.24
	Monoamino N	12.22	9.83	10.41	10.72
,, ,,	Total N	100.00%	100.00%	100.00%	100.00%
	Amide N	23.00	23.32	18.52	13.17
	Humine N	1.70	1.81	1.34	1.45
	Diamino N	7.69	17.56	16.50	14.75
	Monoamino N	67.61	57.31	63.64	70.63

It is certain that the three protains newly obtained are completely different in nature from the hordein obtained by Osborne, who states that it is the homogeneous substance.

The so-called denaturation in the course of the preparation of samples which gives the protein more or less changing of properties demands consideration, but these questions will be elucidated in a future work.

VI. Summary.

1. The alcohol-soluble proteins were separated from the new and old naked barley and their nature was studied from the physico-chemical and pure chemical view points.

Since two out of the new proteins separated were found to be the same, the remaining two proteins were different in their nature from the first two.

2. The alcohol-soluble proteins of the naked barley seem, therefore, not to be a homogeneous substance as in the case of hordein of the common barley obtained by Osborne. In addition, it seems that the proteins in the old and new naked barley were somewhat different in the nature.

3. The above results seem certain. However still further research will be required as to the identity of the alcohol-soluble proteins of the common barley with those of the naked barley, and as to the nature of the proteins in relation to the age of the grains and to the so-called denaturation in the course of the preparation of samples which gives the protein more or less change of properties.

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