ON THE CHANGE OF BARLEY PROTEIN IN STORAGE AND GERMINATION

By

EIJI TAKAHASHI AND KIYOSHI SHIRAHAMA

(With 5 Text Figures)

Introduction

We have reported lately some result[14] of an investigation on the proteins of naked barley. Observing the distinct difference between the proteins separated from old and new grains in their chemical and physical properties, the idea came to us that some changes naturally occure during storage.

Though many investigations have been made hitherto of the chemistry of cereal proteins, almost no attention has been given to the period of its storage, and the study of them is generally confined to general analysis.

Research on the change of protein during the time of storage, it appears, is very important, not only in obtaining fundamental ideas about the property of cereal proteins, but also from the view point of chemical industries consuming cereals as their material,

The change of protein by germination must be also an important problem to be investigated by the brewing industry. The flavour, gas holding power, foaming, nutritive value, preservative power and cloudiness after pasteurization of beer are all affected by the properties of proteins present in the wort.

This new basic research upon the protein in seeds may throw some light upon the solution of these problems. With such ideas in mind we intended to study the phenomena taking place in storage and germination.

Though naked and brewing barley are somewhat different in their protein contents and their natures, they are grains of the same species and the tendency of changes of their proteins at the same condition may be taken as quite equal on the whole. The result of the present inves-

[Jour. Facul. Agr., Hokkaido Imp. Univ., Sapporo, Vol. XXX, Pt. 2, March, 1931]

tigation may reasonably be applied to the case of brewing barley as well.

I. The Change of Proteins in Storage

The change of naked barley protein during three years storage from 1926 to 1929 was investigated. In this chapter the transformation of various kinds of protein and the change of hordein which is the most important one among barley protein, will be described.

The barley used in this research was the product of Hokkaido in 1926. The name of the cultivated variety thus used is "marumi" (*Hordeum sativum* JESS. form *marumi*). After being dried well in the sunlight it was stored in a calorimeter chamber where the variation in temperature and moisture was slight. The analysis was made in September of 1926, 1927, 1928 and 1929. The sample was prepared by grinding the seeds and passing them through a 0.5 m.m. sieve.

Moisture, ash and total nitrogen contents of barley flour were as follows:—

1926

Moisture		Ash		Total Nitrogen	
Sample	Moisture	Sample	Ash	Sample	N
g	%	g	%	g	%
0.9970	13.82	0.8646	1.80	0.4120	2.3250
1.0042	13.78	0.8658	1.92	0.3150	2.2931
1.0046	13.98	0.8521	1.89	0.3567	2.2816
				0.3002	2.3010
(Ave. value)	13.84	(Ave. value)	1.87	0.4020	2.2358
				(Ave. value)	2.2873

Total nitrogen as moisture and ash free.....2.720

1927

Moisture		Ash		Total Nitrogen	
Sample	Moisture	Sample	Ash	Sample	N
g	%	g	%	g	%
0.7862	10.57	0.9721	1.88	0.3068	2.6551
0.6234	10.70	1.0125	1.90	0.3250	2.6414
0.7011	10.80	0.9631	1.89	0.3578	2.6813
				0.3002	2.6430
(Ave. value)	10.69	(Ave. value)	1.89	0.4101	2.6204
				(Ave. value)	2.6483

Total nitrogen as moisture and ash free.....3.29

1928

Moisture		Ash		Total Nitrogen	
Sample	Moisture	Sample	Ash	Sample	N
g	%	g	%	g	%
0.5789	12.95	1.0000	1.72	0.3425	2.5223
0.6234	12.80	0.9823	1.72	0.3072	2.5012
0.7203	12.89	0.9975	1.72	0.3787	2.5232
				0.4123	2.5203
(Ave. value)	10.88	(Ave. value)	1.72	0.4251	2.5345
				(Ave. value	2.5203

Total nitrogen as moisture and ash free.....2.951

1929

Moisture		Ash		Total Nitrogen	
Sample	Moisture	Sample	Ash	Sample	N
g	%	g	%	g	%
0.8438	14.174	1.3806	1.61	0.7812	2.4028
0.8814	14.250	1.4619	1.57	0.5235	2.4009
0.9012	14.212	1.5423	1.60	1.0023	2.3598
				0.9786	2.4602
(Ave. value)	14.212	(Ave. value)	1.59	0.9912	2.4020
				(Ave. value)	2.4019

Total nitrogen as moisture and ash free.....2.852

A. Transformation of the Various Kinds of Proteins

(1) Nitrogen of Five Kinds of Proteins

For nitrogen determination of five kinds of proteins, 300 c.c. of water (10°C) was added to 10 grams of flour, shaken one hour in machine. After precipitation the super solution was removed and again extracted for half hour with 150 c.c. of water. The extracts were combined and centrifuged. The clear solution was taken into 500 c.c. measuring flask filled to 500 c.c. from which 5 c.c. was taken to determine the nitrogen value of the water soluble by means of micro KJELDAHL's method. The residue was extracted just as above with 300 c.c. and 150 c.c. of 10 % NaCl solution, filled to 500 c.c. with 10 % NaCl solution and 5 c.c. was taken to determine the nitrogen of 10 % NaCl solution soluble. The residue was washed several times with water, then extracted with 70 % alcohol as above and the alcohol soluble protein nitrogen was determined. Finally the residue was gathered in filter

paper, dried over night, treated with 0.2 % NaOH solution and from which alkali soluble protein nitrogen was determined.

Subtracting the four nitrogens mentioned above from the total nitrogen the insoluble protein nitrogen was found. The analytical result was as follows. (The following figures are milligrams of nitrogen in ten grams of flour.)

1926

	(I)	(II)	Ave. of (I) and (II)
	mg	mg	mg
Water soluble	32.9400	33.6023	**33.2832**
	32.9400	33.5946	
	33.3975	33.6820	
	33.9400	**33.6263**	
10 % NaCl soluble	25.6200	26.5805	**29.0805**
	25.6200	26.4525	
	26.0775	26.5720	
	25.6200	**26.5350**	
70 % Alcohol soluble	41.6325	39.8025	**39.5738**
	39.3450	39.8025	
	39.5738	38.2013	
	39.3450	**39.8025**	
0.2 % NaOH soluble	71.14125	71.14125	**74.1413**
	71.14125	71.14125	
	67.02375	67.9387	
	71.14125	**71.14125**	

1927

	(I)	(II)	Ave. of (I) and (II)
	mg	mg	mg
Water soluble	29.7854	30.5758	**30.2134**
	29.7854	30.6122	
	30.4524	30.7362	
	29.7854	**30.6414**	
10 % NaCl soluble	31.8555	31.2188	**31.5448**
	31.8555	31.1982	
	29.0313	31.2903	
	31.8555	**31.2341**	

	(I)	(II)	Ave. of (I) and (II)
	mg	mg	mg
70 % Alcohol soluble	56.8572	56.3422	56.6535
	56.8572	56.1782	
	58.2345	56.8240	
	56.8572	**56.4498**	
0.2 % NaOH soluble	86.0023	86.1324	86.1047
	86.1054	86.1052	
	86.0864	86.1965	
	86.0647	**86.1447**	

1928

	(I)	(II)	Ave. of (I) and (II)
	mg	mg	mg
Water soluble	34.7940	34.7470	34.7745
	34.8050	34.7470	
	34.8070	——	
	34.8020	**34.7470**	
10 % NaCl soluble	26.8835	26.8235	26.8875
	26.9005	26.9015	
	26.8995	26.9175	
	26.8945	**26.8805**	
70 % Alcohol soluble	40.9875	40.9945	41.0483
	41.0215	41.0235	
	41.3253	40.9390	
	41.1111	**40.9855**	
0.2 % NaOH soluble	90.8586	92.7710	91.9911
	91.8830	92.4010	
	91.7980	92.2350	
	91.5132	**92.4690**	

1929

	(I)	(II)	Ave. of (I) and (II)
	mg	mg	mg
Water soluble	30.6012	31.0020	30.8016
	30.6012	31.0020	
	30.5880	——	
	30.6012	**31.0020**	

	(I)	(II)	Ave. of (I) and (II)
	mg	mg	mg
10 % NaCl soluble	26.2122	27.0140	**26.6131**
	26.2122	27.0140	
	——	27.0140	
	26.2122	**27.0140**	
70 % Alcohol soluble	31.4000	30.6124	**31.0063**
	31.4000	30.6014	
	31.3988	30.6112	
	31.4000	**30.6125**	
0.2 % NaOH soluble	121.0222	121.8146	**121.4467**
	121.0012	121.8022	
	121.1200	121.8264	
	121.0717	**121.8216**	

Average number is shown by gothic.

(2) Water Soluble Protein Substances

Albumins, proteoses. peptone, amino acids and ammonia etc., are all soluble in water. So if we can determine the quantity of these substances separately, it must be very convenient to see the transformation of water soluble nitrogenous substances.

HILLER and VAN SLYKE (6) investigated the action of various precipitants toward the aqueous solution of partially digested proteins and found that trichloroacetic acid (2.5 %) precipitate the higher class of protein while tungstic acid (1 %) precipitated the intermediate products as completely as possible. For the present research, we modified HILLER and VAN SLYKE's method as follows:—

450 c.c. was taken from 500 c.c. of the water extract mentioned in the previous section (1), 25 c.c. of 50 % trichloroacetic acid was added to it and made up to 500 c.c. with water, well shaken, filtered after a little while and the nitrogen of the filtrate was determined (a). 250. c.c. of the filtrate therefrom were treated with 50 c.c. of 2/3 N sulfuric acid solution and 50 c.c. of 10 % sodium tungstate solution, filled up to 500 c.c., filtered, and the nitrogen of the filtrate was determined (b). Then the nitrogen of the precipitate caused by the addition of trichloroacetic acid was estimated by subtracting (a) from the water soluble total nitrogen, and that precipitable by tungstic acid by subtracting (b) from (a).

The milligrams of nitrogen calculated to ten grams of flour will be shown as follows:—

1926

	(I)	(II)	Ave. of (I) and (II)
	mg	mg	mg
In the filtrate from trichloroacetic acid treatment	19.5714	18.8098	19.1906
	19.5714	18.8098	
	20.3345	18.0020	
	19.5714	**18.8098**	
In the filtrate from tungstic acid treatment	12.2010	11.9468	12.0739
	12.2010	11.9468	
	8.8965	13.9803	
	12.2010	**11.9498**	

1927

In the filtrate from trichloroacetic acid treatment	17.2035	17.6572	17.4023
	17.1021	17.6680	
	17.0712	17.7118	
	17.1256	**17.6790**	
In the filtrate from tungstic acid treatment	12.9075	13.8395	13.1435
	12.9532	13.8395	
	13.1018	14.4230	
	12.9875	**13.8395**	

1928

In the filtrate from trichloroacetic acid treatment	12.5210	12.4930	12.5070
	12.5210	12.4930	
	12.5210	12.4930	
	12.5210	**12.4930**	
In the filtrate from tungstic acid treatment	10.9520	11.1560	11.0540
	10.9520	11.1560	
	10.9520	11.1560	
	10.9520	**11.1560**	

1929

In the filtrate from trichloroacetic acid treatment	23.8424	24.0022	23.9223
	23.8040	24.0022	
	23.8424	——	
	23.8424	**24.0022**	
In the filtrate from tungstic acid treatment	20.9212	21.5026	21.2119
	20.9212	21.5026	
	——	21.5026	
	20.9212	**21.5026**	

Average number is shown by gothic.

The average values of the preceding results were calculated by the method above cited. The nitrogen of the precipitates through trichloroacetic acid and tungstic acid was found to be as follows:—

	1926	1927	1928	1929
	mg	mg	mg	mg
N of the trichloroacetic acid precipitate	14.0926	12.8111	22.2675	6.8793
N of the tungstic acid precipitate	7.1167	3.9888	1.4530	2.7104
N in the filtrate from the tungstic acid precipitate	12.0739	13.4135	11.0540	21.2119

(3) Tendency toward Transformation of Various Kinds of Proteins

Now assuming that there is no natural escape of nitrogen, the ratio of five protein nitrogens to the total nitrogen will be shown in Table 1.

Table 1. *The transformation of various kinds of protein nitrogen in storage*

	1926	1927	1928	1929
Total nitrogen	100.0	100.0	100.0	100.0
Water soluble N	14.6	11.4	13.8	12.8
Albuminous[1] N	6.2	4.8	8.8	2.9
Proteose and peptone[2] N	3.1	1.5	0.6	1.1
N of the amino acid and the others[3]	5.3	5.1	4.4	8.8
10 % NaCl soluble N	11.4	11.9	10.7	11.1
70 % alcohol soluble N	17.3	21.4	16.3	12.9
0.2 % NaOH soluble N	31.1	32.5	36.5	50.6
Insoluble protein N	25.6	22.8	22.6	12.6

For convinience, nitrogen of the protein substance precipitated by various precipitants are indicated as seen in table 1.

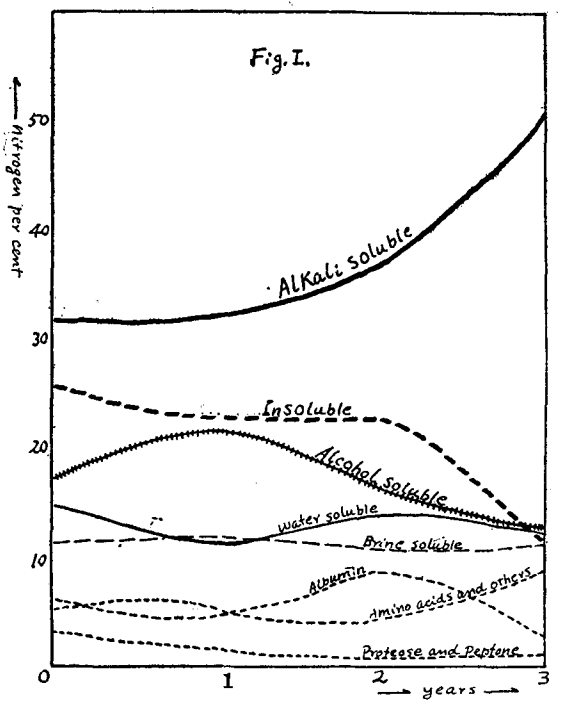
1) Nitrogen of the precipitate caused by the additon of tricloroacetic acid.

2) The ones precipitated by tungstic acid.

3) The ones not precipitated by the above two precipitants.

The above table shows the total transformation of various kinds of protein. It is more clearly shown in the curves of Fig. I.

As seen in the above result, a marked transformation is made during storage except for the NaCl soluble. The change of the NaCl soluble is least, in one year showing a slight tendency to increase, afterward at some time decreasing and again increasing. But it seems to have a somewhat significant meaning among the transformations of other proteins.



The tend of changes of the alcohol soluble and the alkali soluble seems to be in a very close relation with those of the insoluble and the water soluble. In one year's storage, as the alcohol soluble increases greatly (beside this, the alkali and NaCl soluble also increase slightly), the insoluble and the water soluble (comparatively higher proteins) decrease. In one year to two years, a decreasing of the alcohol soluble and an increasing of almost equal degree of the alkali soluble are seen while the water soluble (higher protein) increases markedly; the insoluble shows almost no change and the NaCl soluble slightly decreases.

In two years to three years, the alkali soluble is greatly increased; opposed to this, the insoluble and the water soluble (higher protein) are decreased and beside these a slight decrease of the alcohol soluble and an increase of NaCl soluble are seen.

Among the transformations, the lower amino acids and the others decrease slightly in the first two years and increase thereafter. These points will be discussed in later pages.

B. The Change of Hordein

In a former section, the quantitative change of proteins which takes place in storage was explained. How they change themselves chemically with hordein which is the essential protein in the barley and easily separable comparatively pure among other sorts was next investigated.

(1) Separation of Hordein

The separation of hordein was carried out in September each year. As to the preparation of hordein A and B the details are here omitted as it has already been reported[14]. The different point from the previous report is that no aceton was used to remove water and lipoid, and that brine water was used to recover the hordein B. On describing the outline of the separation, the hordein which is separated by OSBORNE's method was dissolved in a small quantity of alcohol, poured into absolute alcohol by which hordein A is separated in the form of a coagulated mass, while B remained as a milky solution. To this solution, a very small quantity of brine water was added, by which the hordein B was precipitated completely. The precipitate was separated and dried, the yield was as follows:—

	Amount of barley flour	yield of hordein		per cent to flour		per cent of total yield (A+B)
		A	B	A	B	
1926	2.0 kg	52.48 g	4.60 g	2.64 %	0.23 %	2.87 %
1927	0.5	11.98	4.61	2.40	0.92	3.32
1928	1.0	9.19	1.99	0.92	0.20	1.12
1929	1.0	11.00	0.40	1.10	0.04	1.14

As seen in the above result, the percentage of the total yield is increased in one year's storage. This fact agrees well with the experiment described in the former section. But observing the yield of hordeins A and B separately, the percentage of A is gradually decreased every year, while B is increased in one year and afterward decreased. On the whole, from the above fact, the increased amount of hordein in one year's storage appears to be due to the increasing of hordein B. However, in this report, the experiment upon A was only described as B is now under study which will be reported later.

(2) Water-Keeping Power

Before determining the moisture, pulverized hordein was placed in an

evacuated sulphuric acid desiccator for two weeks. The moisture content under this condition might be taken to signify the degree of water-keeping power of protein. For determination, the sample was dried at 105–110°C. for 6 hours and weighed.

The result is as follows:—

	Sample	Moisture	Moisture per cent	Average
1926	232.120 mg	10.445 mg	4.50%	4.50 %
	198.130	8.916	4.50	
1927	149.970	5.000	3.334	3.30
	100.140	3.280	3.275	
1928	410.140	19.960	4.87	4.88
	382.990	18.776	4.89	
1929	224.080	17.140	7.66	7.63
	275.220	20.870	7.60	

As seen in the above, the water-keeping power of hordein changes gradually. After the first year the power decreases and afterward increases.

The like phenomenon is also found in the moisture contents of barley flour. A close relation seems to be present in both cases.

The comparisons are as follows:—

	1926	1927	2928	1929
Hordein	4.50%	3.30%	4.88%	7.63%
Barley flour	13.84	10.69	12.88	14.21
Hordein	100	73	108	170
Barley flour	100	77	93	103

The moisture content of hordein may be considered to have bearing upon a certain affinitive power of the protein for water. The moisture of barley flour may naturally be affected by this property as it is seen in the above table.

(3) Ash and Total Nitrogen

The ash content of hordein was determined as follows:—

	Amount of sample mg	After burned mg	Ash per cent %	Ave. value (% in moisture free)
1926	725.130	0.790	0.109	0.109
	527.520	0.522	0.099	
1927	557.270	1.010	0.179	0.185
1928	793.130	1.317	0.166	0.175
1929	372.170	0.460	0.124	0.155
	584.200	0.940	0.161	

1926	1927	1928	1929
0.109 %	0.185 %	0.175 %	0.155 %
100	170	161	142

Ash per cent increased in one year but afterwards decreased gradually.

The total nitrogen was then determined by KJELDAHL's method as follows:—

	Sample mg	Nitrogen mg	Per cent. %	Ave. %	Per cent in moisture and ash free sub. %
1926	8.715	1.375	15.78	15.74	16.50
	7.112	1.117	15.71		
	9.005	1.417	15.74		
	5.980	0.941	15.73		
	10.200	1.607	15.75		
1627	9.588	1.537	16.03	15.92	16.50
	8.460	1.347	15.93		
	13.800	2.182	15.81		
1928	8.212	1.306	15.90	15.91	16.76
	7.513	1.193	15.88		
	10.125	1.615	15.95		
1929	18.160	2.837	15.62	15.52	16.83
	7.260	1.125	15.50		
	13.550	2.092	15.44		

1926	1927	1928	1929
16.50	16.50	16.76	16.83
100	100	102	102

The change of total nitrogen is almost imperceptible but a somewhat increasing tendency is noticed each year.

(4) The Proportion of Amino Acid Nitrogen

Hordein was hydrolyzed with hydrochloric acid and analyzed by VAN SLYKE's method except that cystine was analyzed by OKUDA's meteod[10]. The analytical results of the nitrogen ratio to protein and to total nitrogen are as follows:—

Table 2. *The Change of nitrogen ratio to protein*

	1926	1927	1928	1929
Amide N	4.082 %	3.984 %	4.063 %	4.231 %
Insoluble humin N	0.111	0.135	0.264	0.099
Soluble humin N	0.055	0.231		0.170
Total basic N	2.847	4.432	3.000	3.145
Free amino N	1.107	0.994	1.079	0.508
Arginine N	1.026	0.894	1.154	1.002
Histidine N	1.456	4.151	1.584	2.828
Cystine N	0.052	-6.13	0.073	0.075
Lysine N	0.313		0.189	-0.760
Total N in filtrate from bases.	9.540	8.358	9.410	9.203
Free amino N	7.669	8.032	7.694	8.300

Table 3. *The change of nitrogen ratio to the total nitrogen of hordein*

	1926	1927	1928	1829
Ammonia N	24.75 %	24.15 %	24.24 %	25.13 %
Insoluble humin N	0.67	0.82	1.56	0.59
Soluble humin N	0.34	1.40		1.01
Total basic N	17.26	26.86	17.90	18.69
Free-amino N	6.71	6.02	6.44	3.02
Non-amino N	10.55	20.84	11.46	15.67
Arginine N	6.22	5.42	6.89	5.95
Histidine N	8.82	25.17	9.45	16.80
Cystine N	0.32	-3.73	0.44	0.45
Lysine N	1.90		1.13	-4.51

	1926	1927	1928	1929
Total N in filtrate from bases	57.80 %	50.67 %	56.15 %	54.68
Free-amino N	46.48	48.69	45.91	49.32
Non-amino N	11.32	1.98	10.24	5.36

In the above table, amide and humin nitrogen show relatively regular values, however great differences among figures below total basic nitrogen are seen. This cause, as stated in the next chapter upon germination, depends upon the fact that the protein contains much more proline, as compared with other various amino acids, and upon analysis by VAN SLYKE's method, the proline was precipitated together with the phosphotungstates of basic amino acids. By these facts, the high values of histidine and arginine and the low value of lysine are shown in the result. The precipitation of proline seemed not alike by the proccess of analysis.

We must consider now the two groups of nitrogen in hordein molecule-that is, primary and secondary types. These groups in this protein are thought to correspond to free amino and no-namino on the whole.

Thus the sums of the free amino or non-amino nitrogen in basic and non- basic fractions are as follows:—

	1026	1927	1928	1929
Sums of free amino nitrogen (a)	53.19 %	54.71 %	52.35 %	52.34 %
Non-amino nitrogen (b)	21.87	22.82	21.70	21.03
(a)	100	102	98	98
(b)	100	104	99	96

From the above table it is observed that the primary and secondary nitrogen in hordein molecule increase in one year's storage and decrease afterward. Such changes are of interest in connection with the humin change. According to the recent investigation humin is, in simple protein, formed principally from tryptophane, tyrosine, cystine and lysine, the tryptophane forming an insoluble humin, the tyrosine a soluble.

These two humins in our result increase in one year's storage and decrease afterward just like the change of primary and secondary amino nitrogen.

Taking these points into consideration, it seems that hordein molecule becomes more complex in one year and afterwards becomes less complex. Quite contrary to the above changes, amide nitrogen decrease in one year and increase afterward.

A close relation seems to exist between these changes. The increase of the latter give rise to the decrease of the former.

The change of cystine has a tendency toward gradual increasing.

(5) A Linkage of Sulphur Nucleus

Cystine and the total sulphur in hordein were analyzed, the former by OKUDA's method and the latter by DENIS-BENEDICT's method. The results were as follows:—

	1926	1927	1928	1929
Cystine sulphur	0.31 %	0.32 %	0.33 %	0.46 %
Total sulphur	0.63	0.62	0.63	0.63
Cyst. S per cent. to total S.	49 %	50 %	52 %	73 %

As seen in the above results, the sulphur nucleus in hordein is clearly changed in storage into the disulphide linkage, especially it occurs in 2–3 years very conspicuously. From the above view points, possibly it may be thought that, besides cystine, another sulphur linkage is present and changed into disulphide linkage.

(6) Peptization

Hordein solution in alcohol-water mixture, was made to appear milky turbid by changing the alcohol concentration and temperature as was reported in a previous paper.[14)15)] Recently DILL and ALSBERG[3)] found also this phenomena in gliadin. They called this phenomena the critical peptization of gliadin and the temperature at which turbidity appears the critical peptization temperature.

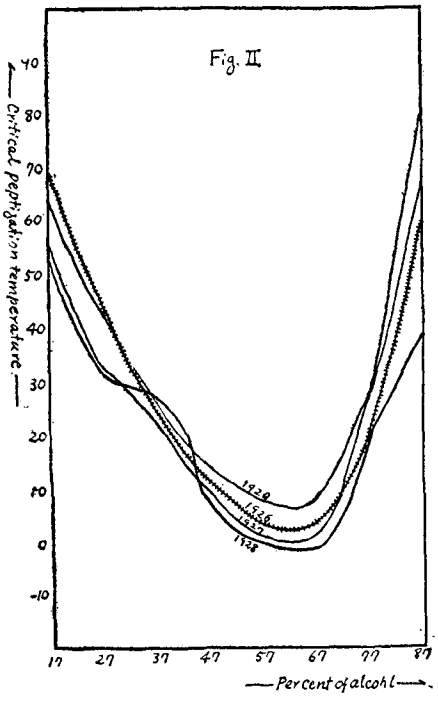
In the present experiments the critical peptization temperature (°C) was measured in ethyl-alcohol and water mixture by means of the

method previously described*.

The result was as follows:—

The change of critical peptization of hordein in storage

Preservation Period \ % of alcohol	17	27	37	47	57	67	77	87
1926	69	43	24	11	3	4	21	60
1927	56	33	23	9	1	2	28	67
1928	53	31	27	8	0	-1	21	39
1929	64	43	26	14	8	8	29	80



We may observe the above change of critical peptization within the following three zones.

Crit. peptization in 17–47 % alcohol solution. Hordein peptization gradually and slightly increased after 0–2 years but decreased after 2–3 years storage.

It is noticed here that the crit. peptization curve is seen at first concave, then convex, more convex and again concave.

Crit. Peptization in 47–67 % alcohol solution. Peptization increased gradually after 0–2 years but greatly decreased after 2–3 pears.

Crit. Peptization in 67–87 % alcohol solution. It decreased at 0–1 year, increased after 1–2 years and again decreased after 2–3 years.

*By the author's method one-tenth gram (calculated as anhydrous substance) of protein is taken into the test tube and dissolved completely in 10 c.c. of 70 % alcohol.

Each 1 c.c. of above solution is added to each 9 c.c. of alcohol-water mixture of various concentrations, being previously prepared in test tubes.

The above test tubes were then individually cooled in ice water or warmed in the water with the thermometer inserted in the tube 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 critical peptization curve is obtained.

On the whole the peptization of hordein increases gradually and slightly during the first two years but decreases greatly after two years.

(7) Tendency of the Change of Hordein in storage

The total yield of hordein from barley flour increases in the first year and afterwards decreases. In detail, hordein A gradually decreases after storage but hordein B increases in the first year and afterwards decreases. Such being the case, the increase of the total yield of hordein in the first year appears to depend upon the increase of B. In the preparation of hordeins, it is noticed that the filtration of alcoholic extract solutions from barleys gradually becomes difficult as the storage proceeds, i. e., the filtration velocity decreases and a clear solution is obtained by repeated filtrations. The nature of hordeins must be changed year by year.

The water-keeping power decreases in the first year and afterwards increases. Ash content increases in the first year, afterward decreases. The change of total nitrogen is not clear but a tendency of gradual decrease is shown.

By VAN SLYKE's analysis, the total free amino nitrogen increases in the first year, afterwards gradually decreases. The change of humin is also the same as above. Amide nitrogen decreases in the first year and afterwards increases. Cystine increases gradually.

The ratio of cystine sulphur to the total sulphur increases gradually in slight amount during the first two years but afterwards increases greatly.

Peptization increases little by little in two years but afterwards decreases greatly. Also, in the critical peptization curve, a peculiar change is found in the neighbourhood of 37 % alcohol concentration.

From the above results, the change of hordein in storage can be observed, divided into chemical and physical, and a close relation between them is also seen to exist. In one year's storage, the molecule of hordein seems to become more complex and afterwards less complex. Characteristics of their physical properties appear to be inevitably produced, accompanied by these chemical changes. It is suggested by the relation between the sulphur nucleus and the peptization.

II. The change of Proteins in Germination

In this chapter the transformation of the various kinds of protein and the change of hordein in germination will be described. Many investigations of the former have been reported up to this time. However, they

are mostly limited to the water soluble protein only, treated with various precipitants. But recently WINDISH[16] has reported an investigation of the change of barley protein. This report can be regarded as a new research from the side of dispersion of protein particles which occurs in the course of brewing. We have investigated, not only the water soluble, but also the other various proteins to see the change of proteins tracing their transformation systematically.

On the change of hordein in germination several reports have been published also since OSBORNE's[11] first reports in 1896, but the results of these investigations do not agree well with each other. We have tried this experiment to clear these points and also to see the change of new hordein which appeared turbid in strong alcohol milky, as has been reported in a previous report.[14]

A. Transformation of Various kinds of Proteins

(1) Preparation of Malts

The naked barley stored for one year was divided into four groups weighing 150 grams each.

They were washed and soaked in water for 24 hours (sometimes the water was renewed) then spread in boxes kept in a thermostat of 25–27°C, covered with wet cloths. After 24, 48, 72 and 120 hours each group was taken out, dried for 24 hours at 60°C., and powdered by a mill. On the other hand the original non-treated barley flour was prepared as a control. These various samples are denoted in the following by these signs.

Original grain	0
Malt of 24 hours growth	24
Malt of 48 hours growth	48
Malt of 72 hours growth	72
Malt of 120 hours growth	120

The yield of malt and moisture, ash and total nitrogen of the samples were as follows:—

	Yield of dry malts.	Yield calculated to 100 grams of original grain.
0	150 gm	100.00 gm
24	130	86.67
48	127.5	85.00
72	125.5	83.67
120	115.0	76.67

The yield of malt and moisture, ash and total nitrogen of the samples are as follows:—

	Yield of dry malts.	Yield calculated to 100 grams of original grain.
0	150 gm	100.00 gm
24	130	86.67
48	127.5	85.00
72	125.5	83.67
120	115.0	76.67

Ash, moisture and total N of the sample

	Moisture		Ash		Total N		Total N free from moisture & ash
	10.57%		1.88%		2.6551%		
					2.6414		
0	10.70	10.69%	1.90	1.89%	2.6813	2.6483%	3.029%
					2.6430		
	10.80		1.89		2.6204		
	6.13		2.01		2.7900		
24	6.00	6.11	2.05	2.03	2.7624	2.7812	3.027
	6.20				2.7912		
	6.75		2.07		2.8556		
48	6.67	6.55	2.07	2.07	2.8056	2.8344	3.101
	6.23				2.8420		
	7.03		2.11		2.8952		
72	8.01	7.83	2.07	2.09	2.8631	2.8843	3.202
	8.45				2.8946		
	9.46		2.20		3.0012		
120	9.07	9.17	2.26	2.23	2.9759	2.9810	3.365
	8.98				2.9659		

From the above results the consumption ratio of organic matters by the growth of malts may be calculated as follows:—

	(sample)
100.00 g (1−0.1069+0.0189)=87.42 gm	0
86.67 (1−0.0611+0.0203)=79.62	24
85.00 (1−0.0655+0.0207)=77.67	48
83.67 (1−0.0783+0.0209)=75.37	72
76.67 (1−0.0917+0.0223)=67.93	120

From the above the consumption ratio will be found to be as follows:—

Consumption ratio of organic matters by growth of malts

	Residue consumed	Ratio to original barley	Consumption ratio	Consumption ratio by various periods of growth
0	(87.42 gm)	(100.00 gm)	0 %	
24	79.62	91.08	8.92	8.92 (0–24 hours)
48	77.67	88.85	11.15	2.23 (24–48 ")
72	75.37	86.22	13.78	2.63 (48–72 ")
120	67.93	77.71	22.29	0.51 (72–120 ")

From this table it is evident that the consumption ratio of organic matters by growth of malts was larger in the periods of 0-24 hours and 72–120 hours after the grain was put in the thermostat. Further more, the morphological observation has been made of the growth of malts as is seen in the following tabular synopsis:—

Period of growth	Rootlet	Germ
0—24 hours	(Length) 2—3 m.m. 97 %	little swelled
24—48 "	3 rootlets 18 % 4 " 62 " 5 " 20 "	(Length) 3-5 m.m. 16 %
48—72 "	4 rootlets 10 % 5 " 75 " 6 " 15 "	(Length) 5-6 m.m. 60 %
72—120 "	5 rootlets 20 % 6 " 70 " 7 " 10 "	(Length) 9-12 m.m. 100 %

As is seen in this table, young roots are seen after 24 hours and gradually increase their number. The germ appearing after 48 hours grew to fair length at 120 hours.

(2) Nitrogen of Various Kinds of Proteins

To determine nitrogen of five kinds of protein, soluble in water, 10 % NaCl solution, 70 % alcohol solution, 0.2 % NaOH solution and insoluble nitrogen, the flour was treated by just the same method as stated already in Chapter I. (B). The milligram amount of nitrogen corresponding to 10 g flour will be shown below.

	0	24	48	72	120
Water soluble	29.7854	48.9212	76.0508	91.5116	123 7158
	30.6414	48.6822	75.8570	90.5828	123.2314
		48.4400	75.8570	90.0984	123.4971
	30.2134	48.6811	75.9216	90.7309	123.4814
10 % brine soluble	31.8555	32.9392	34.8768	32.4548	30.5172
	31.2341	32.9200	33.4236	29.0328	30.5172
		32.9704	34.1496	32.4548	31.0016
	31.5448	32.9432	34.1500	32.4548	30 5172
70 % alcohol soluble	56.8572	29.4515	19.4729	18.0197	5.4253
	56.4498	30.4203	19.9573	17.2931	6.3941
		29.1609	19.9573	19.4872	6.8785
	56.6535	29.3012	19.9573	17.6000	6.6313
0.2 % caustic soda soluble	86.0647	93.3923	80.8948	47.9556	66.9472
	86.1447	93.4892	82.3480	48.9244	68.3002
		93.9736	81.8636	48.4400	66.9472
	86.1047	93.5833	81.7021	48.4400	66.9472

Next, the analysis for water soluble protein substances was carried out by the method which has already been described in Chapter I. A. (2).

Milligrams of nitrogen corresponding to 10 g of malt flour is as follows:—

	0	24	48	72	120
Nitrogen in the filtrate treated by trichloro-acetic acid.	17.1256	36.9631	50.9624	60.9287	94.9521
	17.6790	36.1721	49.8812	60.4415	95.6312
		36.1721	50.4527	60.4415	96.0496
	17.4023	**36.2134**	**50.4321**	**60.4415**	**95.5443**
Nitrogen in the filtrate treated by tungstic acid.	12.9875	19.5138	30.1345	35.6543	78.3025
	13.8395	19.5426	30.1040	35.8521	77.3914
		19.2141	30.4023	35.6365	79.2436
	13.4135	**19.4235**	**30.2136**	**35.7145**	**78.3125**

The gothic in the table shows the average number. From the above average values the milligrams of nitrogen of the precipitates caused by the addition of trichloroacetic acid can be calculated. The results of such calculation are shown below.

Table. 4. *Analytical results of various kinds of protein nitrogen in malt*

(milligrams of nitrogen correspondings to 10 g malt flour)

	0	24	48	72	120
Total N	264.83	278.12	283.44	288.44	298.10
Water sol. N	30.2134	48.6811	75.9216	90.7309	123.4814
Albuminous N	12.8111	12.4678	25.4895	30.2894	27.9371
Proteose and Peptone N	3.9888	16.7899	20.2185	24.7270	17.2318
N of the amino acids and the others.	13.4135	19.4235	30.2136	35.7145	78.3125
NaCl sol. N	31.5448	32.9432	34.1500	32.4548	30.5172
alcohol sol. N	56.6535	29.3012	19.9573	17.6000	6.6313
NaOH sol. N	86.1047	93.5833	81.7021	48.4400	66.9472
Insol. N	60.3136	73.5112	71.7090	99.2043	70.5229

(3) Tendency of the Transformation of Various Kinds of Protein

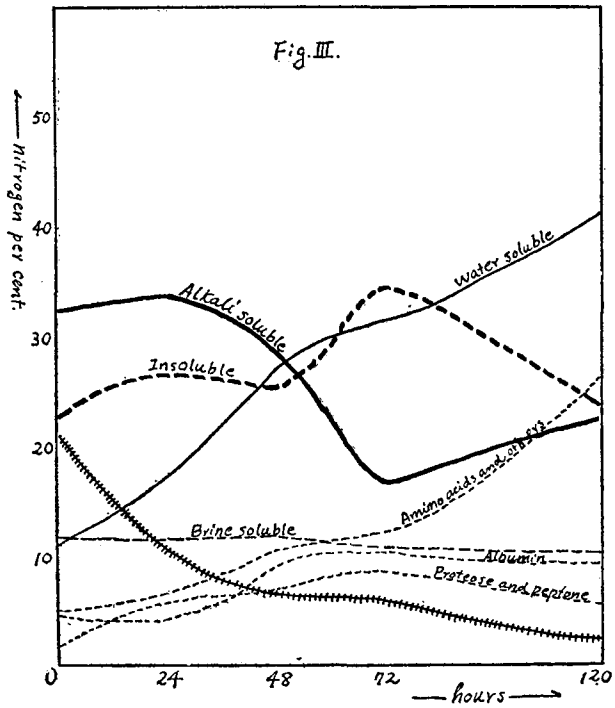
Now assuming that there is no natural escape of nitrogen, the ratio of the five proteins and water soluble protein nitrogens to the total nitrogen will be shown in the following table.

Table 5. *Transformation of various kinds of protein nitrogen*

	0	24	48	72	120
Total N	100.0	100.0	100.0	100.0	100.0
Water sol. N	11.4	17.5	26.8	31.5	41.4
Albuminous N	4.8	4.5	9.0	10.5	9.4
Proteose and pepton N	1.6	6.0	7.1	8.6	5.8
N of the amino acid and the others.	5.0	7.0	10.7	12.4	26.2
10% NaCl sol. N	11.9	11.8	12.0	11.3	10.2
70% Alcohol sol. N	21.4	10.5	7.0	6.1	2.2
0.2% NaOH sol. N	32.5	33.7	28.8	16.8	22.5
Insol. N	22 8	26.5	25.4	34.3	23.7

The above transformation is clearly shown in the curves of Fig. 3.

The most marked transformation is seen in the proteins other than the NaCl soluble. The transformation of the latter is least and no change



is seen at the first stage of germination. It increases somewhat as the germination goes on and then decreases slightly. It will be noticed, however, that this tendency is quite identical with that of the higher protein substances of the water soluble.

Now the tendencies of transformations will be observed separately, grouping together those showing increase and those showing decrease. There will be seen a close relationship between the alcohol soluble and the alkali soluble, and the insoluble and the water soluble.

At the first stage of germination, i. e., 0–24 hours in transformation, the alcohol soluble decreased, while the insoluble and the higher protein of the water soluble increased.

In the second stage, i. e., 24–72 hours, the alkali soluble decreased more rapidly than the alcohol soluble, while on the other hand the insoluble and the water soluble (especially, relatively higher proteins) increased markedly. Moreover, NaCl soluble increased slightly.

In the third stage of germination, i. e., 72–120 hours, the alkali soluble increased, but the insoluble and higher protein substances in the water soluble still kept the decreasing tendency. The alcohol soluble and the NaCl soluble also decreased slightly.

Amino acids and others, the lowest class of the water soluble substances, always increased markedly with the germination, which are quite different from the tendencies of transformations of higher protein substances and the NaCl soluble.

B. The Change of Hordein

In 1896, OSBORNE and CAMPBELL reported first on an alcohol soluble protein in malt. From this investigation, it was thought by them that the alcohol soluble protein in malt, being different in elementary composition from that in the barley grain, a new protein was produced in germination, replacing hordein and they called it "bynin". Afterward, KRAFT opposed OSBORNE and CAMPBELL's opinions and concluded that bynin and hordein are identical.

A more extended research must be made in this field in order to solve this long buried question.

(1) Separation of Hordein from Malts

The malt used for the separation of hordein was prepared by the same method as described in a foregoing section and the sample was prepared from malt of 24, 48 and 72 hours growth (reference to II. A. (1)). Every sample is designated by the following signs:—

Original grain. ..... (0)
Germinated in thermostat for 24 hours after soaking in water. ..... (24)
Germinated in thermostat for 48 hours after soaking in water. ..... (48)
Germinated in thermostat for 72 hours after soaking in water. ..... (72)

The preparation of hordeins A and B was carried out as in the experiment of storage set forth in the preceding chapter.

The yield is as follows:—

Quantity of malts		Yield of hordein		Per cent to flour		Total yield
		A	B	A	B	A+B
Sample	kg	g	g	%	%	%
(0)	0.5	11.98	4.61	2.40	0.92	3.32
(24)	1.5	20.11	6.95	1.34	0.37	1.71
(48)	1.5	16.17	3.69	1.03	0.25	1.33
(72)	1.5	13.43	trace	0.90	—	0.90

It is worthy of notice that the properties of hordein A which is separable as coagulum from alcohol, change gradually according to the growth of malt. Though in cases of (0) or (24), hordein A separates as soft coagulum, in (48) or (72) it separates as a solid mass. Moreover it is important to notice that by (72) hordein A markedly increases its solubility in water while there occurs no more milk turbidity of hordein B in treating with absolute alcohol. For the separation of (72) A the following method was adopted. The malt flour was first extracted with alcohol. When the alcohol solution was evaporated according to the usual method, there remained the syrup containing much maltose. The syrup was cooled next with ice and salt, and the coagulum thereby appeared was separated by decantation. Next, maltose was removed from the coagulum by repeated washing with small quantities of water with ice. The coagulum was dissolved in alcohol and poured into absolute alcohol. Thus hordein A was obtained as a solid mass. That the amount of yield of hordein decreases according to the growth of malt, agrees well with the experiment described in former pages. As to the quantity of hordein A and B, contrary to the case of storage, both decrease in parallel and hordein B separated after 48 hours' growth revealed almost no milk turbidity in absolute alcohol.

(2) Ash, Moisture and Total Nitrogen

To determine the moisture, the refined hordein was put in an evacuated sulphuric acid desicator for 2 weeks.

Next, it was powdered, further placed in a calcium chloride desiccator for one month. So the moisture content under these conditions may be considered to indicate a certain degree of water-keeping power of protein. In determination, the sample was dried at 105–110°C. for 6 hours and weighed. The result was as follows:—

		Sample	Moisture	Percentage	Average
(0)	A	149.970 mg	5.000 mg	3.334 %	3.304 %
		100.140	3.280	3.275	
(0)	B	82.226	2.906	3.534	3.534
		71.960	2.550	3.535	
(24)	A	214.070	9.700	4.531	4.498
		172.900	7.720	4.465	
(24)	B	92.310	3.220	3.488	3.493
		80.580	2.820	3.499	

		Sample	Moisture	Percentage	Average
(48)	A	239.950 mg 158.880	13.066 mg 8.722	5.445% 5.489	5.467%
(48)	B	131.340	4.556	3.468	3.468
(72)	A	157.750 185.270	6.486 7.615	4.111 4.110	4.111

Moisture of hordein

	(0)	(24)	(48)	(72)
A	3.304 %	4.498 %	5.467 %	4.111 %
B	3.534	3.493	3.468	—
A	100	136	165	124
B	100	99	98	—

We can see from the above, the tendency of hordein A increasing up to 48 hours, after that decreasing slightly, while, B shows only a small difference between the figures. That the moisture-keeping power of protein itself increases for some time and decreases afterward, seems to have some significance in biochemistry of plants.

Next, the ash content was determined. The result was as follows:—

		Sample	Ash	Per cent.	% in moisture free state
(0)	A	557.270 mg	1.010 ng	0.179 %	0.185
(0)	B	234.700	1.044	0.445	0.462
(24)	A	738.162	0.980	0.133	0.139
(24)	B	163.730	0.380	0.232	0.240
(48)	A	652.444	0.730	0.113	0.119
(48)	B	172.290 139.778	0.143 0.126	0.083 0.090	0.090
(72)	A	563.820	0.510	0.090	0.094

Ash in hordein

	(0)	(24)	(48)	(72)
A	0.185 %	0.139 %	0.119 %	0.094 %
B	0.462	0.240	0.090	—

	(0)	(24)	(48)	(72)
A	100	75	64	51
B	100	52	19	—

The high content of ash in B may be due, to some degree, to the use of the salt for the precipitation of the protein; but its change is very regular, so that it seems as if the ash exists as an essential ingredient of the protein, as seen in the case of storage. B in comparison with A shows a marked decrease of ash content with its growth.

The result of the determination of total nitrogen was as follows:—

		Sample	N	%	Average	% in ash and moisture free
		mg	mg		%	%
		9.588	1.53702	16.031		
(0)	A	8.460	1.34733	15.926	15.291	16.496
		13.800	2.18150	15.808		
		9.186	1.44947	15 779		
(0)	B	11.600	1.82886	15.766	15.773	16.427
		10.106	1.62944	16.123		
(24)	A	10.736	1.71699	15.993	15.973	16.749
		9.971	1.57594	15.805		
		9.548	1.50054	15.716		
(24)	B	10.300	1.62214	15.748	15.732	16.341
		10.480	1.60993	15.362		
(48)	A	9.620	1.46406	15.219	15.287	16.190
		9.940	1.52486	15.341		
		11.006	1.67565	15.225		
		10.740	1.614 85	15.036		
(72)	A	9.888	1.48352	15.003	15.035	15.694
		10.170	1.53216	15.065		

Total nitrogen of hordein

	(0)	(24)	(48)	(72)
A	16.496 %	16.749 %	16.190 %	15.694 %
B	16.427	16.341	16.396	—
A	100	102	98	95
B	100	99	100	—

Generally, speaking of the change of total nitrogen, hordein A decreases gradually according to its growth while B remains almost unchanged, though the slight increase of A in (24) and the decrease of B in (48) are noticiable.

(3) The Proportion of Amino acid Nitrogen

The change of nitrogens of various forms was next investigated. Pains were taken to make the conditions of analysis all equal, so that seven samples were treated at the same time. To one gram of protein 20 c.c. of 20 % HCl was added, hydrolysed for 30 hours, filtered, insoluble humin nitrogen was determined from the residue. From the filtrate, the equal volumes corresponding to 1 g of protein was taken, its amide nitrogen was determined and then the soluble humin nitrogen was determined with the residue from amide nitrogen.

The filtrate free from amide was treated with phosphotungstic acid. The solution was then put aside for 23 days (evening of Feb. 10th, to morning of March 5th) at room temperature, by some accident, instead of 48 hours as described in VAN SLYKE's method.

Insoluble humin nitrogen was determined with the residue of hydrolysis which was decomposed with sulphuric acid, made to 100 c.c., and from which 5 c.c. was taken and analyzed. The result was as follows:—

Sample		Quantity of hordein used for hydrolysis. In moisture and ash free state	Insoluble humin N	%
		g	mg	
(0)	A	4.187052	0.2845	0.136
(0)	B	2.810880	0.1532	0.109
(24)	A	4.004243	0.2529	0.126
(24)	B	2.791080	0.1289	0.092
(48)	A	3.776031	0.2821	0.149
(48)	B	2.022643	0.0997	0.099
(72)	A	4.145663	0.3308	0.160

Amide nitrogen was then determined with the filtrate from the above insoluble humin.

Milligrams of amide nitrogen corresponding to one gram of hordein in moisture and ash free state are as follows:—

	(0)	(24)	(48)	(72)
	39.875 mg	40.988 mg	39.874 mg	38.059 mg
A	39.803	40.296	39.070	38.219
	3.984 %	4.063 %	3.947 %	3.813 %

	(0)	(24)	(48)	(72)
B	38.044 mg 38.441	38.720 mg —	38.481 mg 38.521	
	3.824 %	3.872 %	3.850 %	

The result of other analysis are shown in table 6 and 7. The correction for solubility of phosphotungstate is omitted.

Table 6. *The change of nitrogen of various forms in germination*

	(0) A	(0) B	(24) A	(24) B	(48) A	(48) B	(72) A
Total N	16.496%	16.427%	16.749%	16.341%	16.170%	16.396%	15.674%
Amide N	3.934	3.824	4.063	3.871	3.947	3.850	3.813
Humin insoluble N	0.135	0.129	0.126	0.092	0.149	0.098	0.159
Humin soluble N	0.231	0.232	0.145	0.226	0.133	0.226	0.121
N of the ppt. by phosphotungstic acid.	4.432	4.614	4.104	4.116	4.043	3.720	3.872
Arginine N	0.894	1.082	0.824	0.923	0.795	0.843	0.735
Histidine N	4.651	4.028	3.855	3.555	3.904	3.226	3.603
Free-amino N	0.994	1.120	0.920	1.054	0.844	1.933	0.859
N in the filtrate from the phosphotungstate	8.358	8.274	8.568	8.400	8.589	8.568	8.274
Free-amino N	8.032	7.682	8.248	7.973	8.323	8.019	7.857
Total	17.144	16.461	17.006	16.706	16.861	16.462	16.239

Table 7. *The change of nitrogen of various forms in germination*

	(0) A	(0) B	(24) A	(24) B	(48) A	(48) B	(72) A	(A)*
Total N	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Amide N	24.15	23.28	24.26	23.70	24.38	23.48	24.30	24.75
Humin insoluble N	0.82	0.66	0.75	0.56	0.92	0.60	0.01	0.67
Humin soluble N	1.40	1.41	0.87	1.38	0.82	1.33	0.77	0.37
N of the ppt. by phosphotungstic acid.	26.86	23.09	24.50	25.19	24.93	22.69	24.67	17.26
Arginine N	5.42	6.59	4.92	5.65	4.91	5.14	4.68	6.22
Histidine N	25.17	24.52	23.02	21.76	4.11	19.68	23.53	8.82
Free-amino N	6.02	6.80	5.47	6.45	5.21	5.69	5.47	6.72
Non-amino N	20.84	21.29	19.03	18.74	19.72	17.00	19.20	10.54
In the filtrate from the phosphotungstate	50.67	50.37	51.16	51.40	53.05	52.26	52.72	57.80
Free-amino N	48.69	46.76	49.24	43.79	51.41	48.91	50.06	46.48
Non-amino N	1.98	38.61	1.92	2.61	1.64	3.35	2.66	11.32
Total	103.90	103.81	101.54	102.23	104.10	100.41	103.47	100.82

* This column is the analytical result abtained in 1926 and mentioned on page 23.

In Table 7 (A),* presents a great difference in comparison. Tracing the cause of such results, we conceived of the precipitability of proline by phosphotungstic acid, which will be explained below:—

On the precipitation of proline by phosphotungstic acid

As stated above, in determination of various kinds of nitrogen by VAN SLYKE' method, when the solutions were allowed to stand for a very long time after being treated with phosphotungstic acid, the nitrogen of the phosphotungstate was greatly increased as compared to the result of hordein (A),* as seen in table 7, and the samples treated, as above revealed so high value of non-amino nitorogen of the basic fraction.

A comparison of non-amino nitrogen value (from table 7) between basic and non-basic fractions is as follows:—

Non-amino N	(A)*	(0) A	(24) A	(48) A	(72) A
(I) In the ppt. by phosphotungstic acid.	10.54	20.84	19.03	19.72	19.20
(II) In the filtrate from the phosphotungstate.	11.32	1.98	1.92	1.64	2.66

From the above, the fluctuation of non-amino nitrogen of (A) A—(72) A to (A)* was as follows:—

	(0)A—(A)*	(24)A—(A)*	(48)A—(A)*	(72)A—(A)*
(I)	+10.30	+ 8.49	+ 9.18	+ 8.66
(II)	− 9.34	− 9.40	− 9.68	− 8.66

The above fluctuating figures are well agreed and they suggest that the substance having non-amino group in (II) fraction have been precipitated in (I) fraction. According to the principle of the VAN SLYKE's method, the amino acids to be precipitated by the phosphotungstic acid are the four bases: arginine, histidine, lysine and cystine. And in the filtrate therefrom primary amino acid and those having secondary nitrogen i, e, the pyrrolidin ring (proline, oxyproline) and indole nucleus (Tryptophane) are to be found. It is reported by OSBORNE and CLAPP[12], who analyzed hordein by the esterification method, the proline content being 13.73% and oxyproline doubtful. 13.73% of proline equals to 1.716% calculated to its nitrogen-that is, just 10.4% to total nitrogen (16.5%) of hordein. From these results it may be said that the large part of non-amino nitrogen in the filtrate from the precipitate of phosphotungstic acid should be nothing other than proline. The value of (0)A—(A)* precipitated during 23 days should be almost total proline.

Recently GORTNER and SANDSTROM [5] made the hydrochloric acid solution mixed artificially with various amino acids; and upon, analysis by VAN SLYKE's method, they found that in the case of addition of much proline in comparison with the other amino acids, not only the histidine, but also the arginine value becomes high, naturally resulting in a very low value of lysine.

Moreover HOFFMAN and GORTNER[7] investigated twelve kinds of prolamine by VAN SLYKE's method and concluded that especially high histidine nitrogen value in hordein is probably due to the precipitation of proline, refering to the SANDSTROM's research. As seen in table 7 we also see the high histidine nitrogen value in our case.

In short, it seems evident that almost the whole of proline is precipitated when the solution is put aside for long time after being treated with phosphotungstic acid. Moreover the precipitation seems to be variable even by the room temperature as seen in the former chapter.

Now observing the change of hordein in germination, all amino acid nitrogen will be divided into two groups as made in our study of storage, the primary and the secondary. They are regarded, on the whole, as a free-amino and a non-amino group.

When each sum of free-amino and non-amino nitrogen in the bases and the filtrate from bases respectively are sought, they are as follows:—

Total sums of	(0)A	(24)A	(48)A	(72)A	(0)B	(24)B	(48)B
free-amino nitrogen	54.71	54.71	56.62	55.53	53.56	55.24	54.60
non-amino nitrogen	22.82	20.95	21.36	21.86	24.90	21.35	20.35
free-amino nitrogen	100	100	103	101	100	103	102
non-amino nitrogen	100	92	94	96	100	86	82

As seen above, the total free-amino nitrogen shows a tendency of gradual increase in hordein A and B, the total non-amino nitrogen has a tendency quite contrary to decrease in A and B. As to the degree of change, B is stronger than A. Next, observing the total free amino nitrogen divided into diamino and monoamino groups, the former decreases in A and B, while the latter increases in both divisions.

Just as above, when we observe also the total non-amino nitrogen divided into the same two fractions, in the diamino fraction the change of diamino itself is not clear because of the high precipitation of proline, but since the free-amino decreases gradually, as shown above, histidine, arginine and lysine ought to have decreased, and really the arginine decreases clearly in A and B. As to the non-amino nitrogen of monoamino, in both A and B, it has a tendency toward decreasing. On the whole, according with the growth of malt, the amino acid nitrogen decreases gradually in the diamino fraction and increases in monoamino fraction. The degree of the change is stronger in B than in A. These changes seem to be closely related with humin as already stated in the chapter on storage. The change of insoluble humin agrees well with that of non-amino nitrogen and the soluble humin also is decreased gradually.

We are aware also that diamino nitrogen decreases in accompaniment with the increase of monoamino and that amide nitrogen has a tendency toward increase.

(4) Linkage of Sulphur Nucleus

Cystine and total sulphur contents are determined by OKUDA's method and DENIS-BENEDICT's method respectively.

Total sulphur and cystine sulphur contents in moisture and ash free state

		Cystine sulphur	total sulphur	Cystine sulphur per cent to total sulphur.
(0)	A	0.32%	0.62%	52%
(24)	A	0.35	0.52	67
(48)	A	0.30	0.78	38
(72)	A	0.35	0.60	58
(0)	B	0.35%	—	—
(24)	B		—	—

As seen, the disulphide linkage in hordein is increased or decreased on the growth of malt.

(5) Peptization

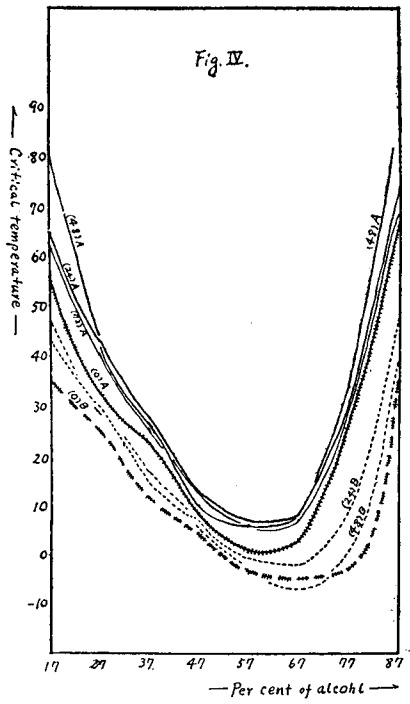
As described in the previous chapter, the same experiment was carried out on the critical peptization of malt hordein.

The results were as follows:—

Critical peptization temperature (°C) of malt hordein A and B

% of alcohol		17	27	37	47	57	67	77	87
(0)	A	56	33	23	9	1	2	28	97
(24)	A	65	43	28	13	7	8	31	74
(48)	A	80	43	27	10	6	8	34	above 80
(72)	A	63	41	26	13	6	7	28	68
(0)	B	35	25	12	4	-3	-5	-3	36
(24)	B	43	30	15	8	0	-2	9	48
(48)	B	47	29	17	6	-3	-7	1	39

As is shown in Fig. IV the malt hordein is less peptized in geneneral. Peptization property of hordein is observed to be decreased by germination. If we compare hordein A and B the latter is more peptizable than the former. In malt hordein we see also such property but decreased peptization is observed generally.



It is not clear whether the deviation which is seen in the critical peptization curve is dependent upon the method of preparation of the samples or upon the influence of germination.

(6) Tendency of Changes of Hordein in Germination

On the growth of malts, the amount of hordein is gradually decreased and the chemical nature of the protein is also greatly changed. The solubility of hordein in water appears gradually to increase and the water-keeping power of protein increases in 0–48 hours but decreases in 48–72 hours. The ash content clearly decreases. Total nitrogen also appears to have a tendency to decrease, though it is not clear.

The proportion of the amino acids in the hordein molecule is also changed markedly as is shown in the results of analysis by VAN SLYKE's method. The total free-amino nitrogen gradually increases, above all, diamino nitrogen, such as arginine, gradually decreases and mono-amino nitrogen, on the contrary, gradually increases, while the total non-amino nitrogen decreases on the whole. Humin nitrogen decreases and amide nitrogen increases. The ratio of cystine sulphur to total sulphur is not constant, increasing or decreasing.

The colloidal nature of hordein, such as peptization is also changed. The peptization power of malt hordein in ethyl alcohol generally decreases more than in barley hordein and the degree is also changed variously by the growth of malts.

The tendencies of the changes described above are all similar in both

hordeins A and B but the degree of the changes is always greater in B than in A.

In short the change of hordein in germination is chemical and physical, as in the case of storage, and has a tendency to become a less complex molecule on the whole.

III. Discussion of the Change of protein

A. Are Hordein and Bynin Identical ?

In 1896, OSBORNE and CAMPBELL[11] isolated from the malt a 75% alcohol soluble protein. They found the difference in elemental composition between this protein and hordein as shown below;—

	Hordein	Bynin
C	54.29 %	55.03 %
H	6.80 %	6.67 %
N	17.21 %	16.26 %
S	0.83 %	0.84 %
O	20.87 %	21.50 %

From the difference of percentage of carbon and nitrogen, they pointed out that these two proteins are not identical. They considered that this malt protein was a new protein produced by germination while hordein itself disappeared. They gave the name "bynin" to their new protein.

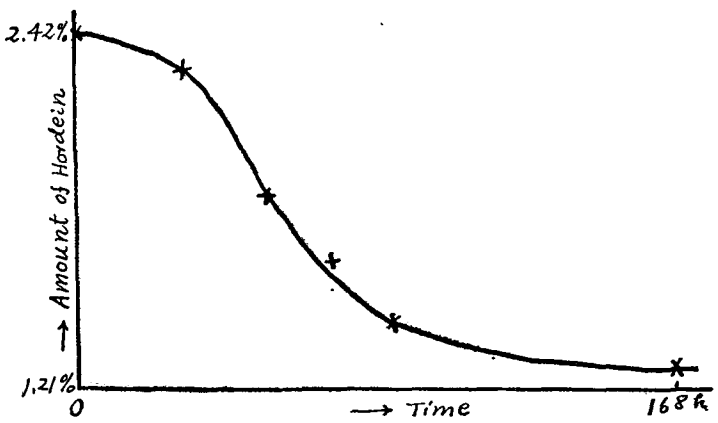
Afterward KRAFT[8] isolated hordein and bynin according to OSBORNE's method and investigated their characteristics. He found the total nitrogen of hordein and bynin to be 16.7 % and 14.2 % respectively, and reported further on their properties. The behavior of bynin and hordein toward alcohol (soluble in 75%, addition of pure water or alcohol gives a turbidity) is similar. He reported further that they behave alike toward pepsin and alkali, the optical activity of hordein $[\alpha]_D = 114.8°$ (in 75 % alcohol), of bynin $[\alpha]_D = 108°$ (in 75 % alcohol). When obtained in such opaque solutions they may be considered identical.

Though KRAFT recognized, as above, certain differences between bynin and hordein, he thought them to be practically identical.

Afterward, LÜERS[9] separated hordein and bynin according to OSBORNE's method and investigated their chemical composition comparatively through complete hydrolysis. He reported the result of his analysis as follows:—

	Hordein	Bynin
Ammonia N	23.00 %	23.55 %
Melanin N	1.70 %	1.67 %
Cystine N	1.58 %	1.63 %
Arginine N	5.00 %	5.23 %
Histidine N	1.33 %	1.09 %
Amino N in Filtrate of Base	54.02 %	52.43 %
Non-amino N in Filtrate of Base	12.49 %	12.39 %
Total	**97.99** %	**99.12** %

And he stated that the difference in the above comparison may have been due to an error in analysis or to the difference of materials from which the samples were prepared. He denied the opinion of OSBORNE and CAMPBELL, stating that with allowance for possible mistakes in his analysis the differences between hordein and bynin can not be acknowledged. So the two proteins are not of different constitution; bynin is nothing more than hordein that remained in germination without being decomposed. Referring also, to KRAFT's experiment, Lüers concluded that no other alcohol soluble proteins can be produced, making his inference from the fact that the quantity of hordein decreases gradually according to growth of malt,



without new protein appearing as seen in the above graph.

Lately, BISHOP[1] investigated the nitrogenous compounds of barley,

and told also about the decreasing of hordein by germination. Now, comparing our results with those of the above authors we find fair agreement in the tendency of changes. For instance, the lower total nitrogen in bynin than in hordein; behaviour toward alcohol and decreasing quantity of hordein by germination; further, the distribution of various nitrogens given by LÜERS, such as low amide and humin nitrogens and high cystine value.

However, as stated already OSBORNE, judging from his elementary analysis, believed in the appearance of new protein in place of hordein, and KRAFT denied OSBORNE's opinion from the view of changing of hordein, insisting that bynin and hordein must be practically identical; and afterward LÜERS investigating the distribution of nitrogen also confirmed the the identity of these two substances, even if he did find some noticeable differences between them.

We think from our experiments that, if the above authors had investigated the properties of bynin at every stage of malt's growth instead of making a simple comparison with hordein and only one sample of bynin, they might have found in it a regular change, though it appeared in a slight degree, without insisting as an experimental error upon the small difference between hordein and bynin.

In short, considered from our result, bynin is now not a simple protein with a constant characteristic property but is considered to be probably a denatured hordein appearing through the course of germination, being soluble in alcohol like its mother substance.

Accordingly, the name "bynin" must be taken hereafter to have a different meaning, signifying all alcohol soluble denatured hordeins influenced by germination.

B. The Significance of the change of Hordein in Storage and Germination

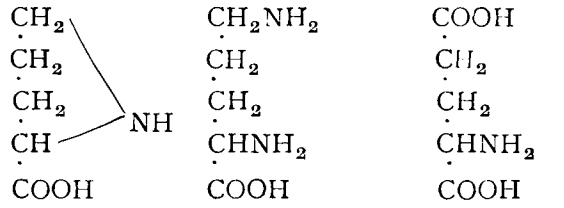
As already mentioned, it was recognized in storage and germination that not only the quantity of hordein but its chemical composition, as well as physical nature, also change.

In one year's storage increase of hordein content is seen; and, upon analysis of hordein, humin nitrogen and secondary amino nitrogen are increased. These facts suggest that the protein molecule becomes more complex.

Quite contrary to the above phnomenon, in germination and in storage

at the intervals of 1~3 years, the reverse phenomenon to become less complex is recognized. As to these changes, some significances are recognized. In germination, secondary amino nitrogen decreases while mono-amino acids and amide nitrogen increase. Such a tendency appears to be very interesting.

DAKIN[4] pointed out already that there is a close relation among proline, arginine, ornithine and glutamic acid or aspertic acid, making his investigation from their changes in animal body as well as from their construction. He also referred to the similarities in their constructions with 5 carbon atoms.



Proline Ornithine Glutamic acid

And in relationship between amide group and mono-amino-dibasic acids, OSBORNE, LEAVENWORTH and BRAUTLECHT[13] have already reported that the amount of ammonia yielded by hydrolysis with acids agreed so closely with that required for amide union with the sum of the glutamic acid and aspertic acid found in a large number of proteins of both vegetable and animal origin, that it seems highly probable that practically all of the ammonia originating from such a combination and one of the carboxyl groups of each molecule of the dibasic acids were thus united with a NH_2 group.

From the above view point, the changes which occur in hordein molecule in germination seem to agree with OSBORNE and DAKIN's statements, i. e., arginine proline etc. are changed to mono-amino-dibasic acids, such as glutamic acid, aspertic acid etc., and at the same time, the content of amide nitrogen in hordein increases.

The changes of ash contained in hordein were also very clearly observed. The increase of its content was seen in storage during the first year but after one year it was seen to decrease; and the latter tendency is also observed clearly in the case of germination.

In storage and germination the sulphur nucleus of hordein is changed to a disulphide form. The change of the ratio of cystin sulphur to total sulphur seems to have some relation to the change of peptization, i. e.,

in 2–3 year's storage those ratios markedly increased while peptization decreased greatly, and in germination those ratios oscillated while peptization was also oscillated. In the critical peptization curves in the case of storage, it is very interesting that the curve shows not only. the degree of peptization but also a peculiar nature of its change. It appears to be a special change due to the colloidal structure of hordein.

Solubility of hordein is also much influenced by storage and germination. A part of hordein becomes the water soluble, brine water soluble, alkali soluble and insoluble.

The water-keeping power in storage decreases as the protein molecule becomes more complex and increases as the molecule becomes less complex while in germination it is increased at 0–48 hours and decreased at 48–72 hours. Observing this from the morphology of malt, this power seemes to increase with the fair development of young rootlet while it inclines to decrease as the young shoot begins to elongate further.

C. The Significance of the Transformation of Proteins in Storage and Germination

The so-called reserve protein, which consists of alcohol soluble, alkali soluble and insoluble proteins, occupies the bulk of the total proteins in barley and undergoes marked transformation in storage and germination. Through our experiment, it has been assertained that the transformations of these proteins have a very close relation with each other, so that it is imposible to discuss it separately. The outline of such transformation can be seen in the curves of Fig. 1 and 2. Three dispositions are naturally observed in it; that is, increase, decrease and nochange. As to the significance of this increase or decrease, different meanings will be considered with proteins higher or lower. For example, alcohol soluble, alkali soluble and insoluble proteins are thought to be in the higher class and the brine water soluble and the water soluble in the lower proteins comparatively. From the increase of the former with the decrease of the latter it may be considered that the former is formed from the latter. If the case is reversed the former is integrated, producing the latter. By the above way of looking at the curves, the phonomena of integration or formation of proteins can be presumed in storage and germination.

In our experiment, in the case of condensation of the one there was always recognized the integration of the other, and vice versa. The evident dispositions are summarized as follows :—

In storage	First stage (0–1 year)	Increase	The alcohol sol. (Alkali sol.)
		Decrease	The insoluble and the water sol.
	Second stage (1–3 years)	Increase	The alkali sol.
		Decrease	The insol. and the water sol.
In germination	First stage (0–72 hours)	Increase	The water sol. and the insol.
		Decrease	The alkali sol. and the alcohol sol.
	Second stage (72–120 hours	Increase	The alkali sol. (nitrogen of amino-acids and others.)
		Decrease	The insol. and the water sol.

The above summary is shown more briefly as follows:—

The insoluble and the water soluble	The alkali sol.	Storage	Increase gradually
		Germination.....	Decrease first and increase next.
	The alcohol sol.	Storage	Increase first and decrease next.
		Germination.....	Decrease gradually.

Remarkable changes are seen except in the brine water soluble. It appears also that the transformation of the insoluble and the water soluble are always accompanied by that of the alkali soluble or the alcohol soluble, suggesting the existence of close relation among these proteins. In other words the transformation of the alkali soluble or the alcohol soluble may be taken as a reversal of that of insoluble and water soluble proteins.

In recent years, Wu and Yen[17] reported on the denaturation of protein and they said, "The first products of the denaturation of the protein are shown to be insoluble, the soluble products being formed only secondarily." Of course, "insoluble" in their report might not be identical with our insoluble protein, however, if we acknowledge the tendency of denatura-

tion of hordein as stated in former pages, it may not be unreasonable to apply such a view to the phenomena of germination and storage.

According to ANSON and MIRSKY's[1] opinions, they say, "When proteins are denatured, they are probably polymerized. Native proteins can be regarded as aggregates of denatured proteins. The denaturation of proteins is a reversible process but the flocculation of denatured protein makes it appear to be an irreversible process." From such view points, it may be considered that a close relation exists between the denaturation and the transformation of proteins in storage and germination.

Summary

In storage and germination of naked barley (*Hordeum sativum* JESS. form *marumi*), the transformation of various kinds of proteins and the chemical or physical change of hordein were studied.

The other proteins, excepting the brine water soluble, made a marked transformation and it was shown that the change of alkali and alcohol soluble proteins are closely related with that of the insoluble and the water soluble proteins.

On the change of hordein, a certain chemical change was recognized in its molecule and a question of whether or not hordein and bynin are identical was made clear.

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