Memoirs of the College of Agriculture, Kyoto Imp. Univ. No. 5. Art. 2. Sept. 1928

On The Iso-electric Point of Glutenin

By

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With 7 tables and 4 text figures

(Received May 15th, 1928)

A. Introduction

The applicable value of wheat flour depends largely on the quantity as well as on the quality of the proteins cotanined therein. For instance, flour strength[1] is affected by the quality of flour-proteins. The hydrolysates of wheat proteins have been thoroughly studied by Tohmas B. Osborne.[2] Therefore, further study may not be needed, in this field, on the wheat proteins.

The wheat kernel contains an average of about 12 per cent. of proteins. In these proteins the substances known as gliadin and glutenin are by far the most essential part of the nitrogenous ingredients. Hence, it may easily be guessed that the quantities and properties of these two kinds of proteins—especially the physicochemical properties —may play a great role in the applicable value of wheat flours. For instance, according to Gortner and Sharp,[3] who studied the physico-

(1) Bailey, C.H., The Chem. Wheat Flour, Chem. Catalog Co., New-York 228 (1925).

(2) Osborne, T.B., The Proteins of the Wheat kernel, Carnegie Institution of Washington, 1907.

(3) Gortner, R.A. & Sharp, P.F., Journ. Phys. Chem. **27**, 481, 567, 674, 771, 942 (1923).

chemical properties of weak and strong flours, the properties of the flours are affected by those of the glutenins. Herewith, the work of a number of experimenteres, who studied these proteins from several samples, is stated. Hall[1] reported in 1905 that the gliadins, which are present in very strong flours and in very weak flours, are not the same substance. However, Wood,[2] after the determination of the amide nitrogen of the gliadins and glutenins which were isolated from the different flours, concluded that the gliadins and glutenins, present in different flours, are respectively identical. Groh and Friedl[3] determined the viscosity, the surface tension and the specific rotation of the gliadin alkali-solution and explained that the wheat kernel contains a single alcohol-soluble protein. On the contrary, Woodman[4] determined the specific rotation of the glutenin alkali-solution and confirmed the theory that the glutenins from the strong and weak flours are not the same. Halton[5] came to still another conclusion. He traced the racemisation curve of the gliadin alkali-solution and confirmed the theory that the gliadins from the various samples are identical with one another. But he fractionated two kinds of glutenin and concluded, after determining the racemisation curves of the alkali-solution of these samples, that they are different substances.

However, Blish and Sandstedt[6] could obtain only one kind of glutenin even after Halton's fractionating method. Therefore they concluded that one kind of glutenin, which Halton fractionated from the glutenin fraction, was a partially racemised glutenin, and therefore it had a different iso-electric point from the genuine glutenin. Cross and Swain[7] have determined the amounts of amino acids in the gliadins and glutenins from various samples, and found that the contents of the amino acids are different respectively in proteins from different flours. However, they attributed this difference to an experimental error and concluded that the gliadin and glutenin from different samples are respectively the same.

Though these conclusions vary, as described above, we can see that the gliadin is of the same composition and of the same property

(1) Hall, A.D., Report of Home-grown Wheat Committee (1905-6), refer the Bailey's Book.
(2) Wood, T.B., Journ. Agri. Sci., **2**, 139, 267 (1907-8).
(3) Groh J. & Friedl G., Biochem. Zeitsch. **66**, 154 (1914).
(4) Woodman, H.E., Journ. Agri. Sci., XII, 231 (1922).
(5) Halton, P., Journ. Agri. Sci., XIV. 587 (1924).
(6) Blish, M.J. & Sandstedt R.M., Cereal Chem., iii, 127 (1925).
(7) Cross R.J. & Swain R.E., Ind. & Eng. Chem., XVI, 49 (1924).

whether obtained from strong or weak flour, whereas glutenin differs in character from one flour sample to another. Hence, study of glutenin not only gives us an interest in the flour and baking chemistry, but also the experimental results will be applicable, with powerful effect to the cultivation and breeding of wheat.

With this intention we will study first the iso-electric point of glutenin. Bailey and Peterson[1] have earlier determined the iso-electric point of gluten with the approximate result of pH value 5.1. Scharp & Gortner[2] showed that glutenin may have an iso-electric range from pH value 6 to 8, since there was a minimum of swelling and imbibition of water by flour through this range of hydrogen ion concentration. Tague[3] worked also in this field observeing the change in hydrogen ion concentration resulting from the addition of a unit quantity of glutenin to Sørensen's phosphate buffer solutions of varying pH. The iso-electric point was regarded as the concentration of hydrogen ions in which the glutenin did not alter that concentration and in which it could flocculate at a maximum, and this was observed to be pH value 6.8 to 7.0. Recently Csonka and Jones[4] fractionated two kinds of glutenins, named α and β, which have the iso-electric point equally at pH value of 6.45.

As described above, the iso-electric point of the glutenin varies according to investigators. This should be a confirmations of the theory that glutenin differs in character from one flour sample to another. However, this can not be a complete confirmation, because the method of preparation of the glutenin and the method of determination of the iso-electric point, which the investigators employed, are not the same. The point of maximum flocculation of a protein so difficult of solution as glutenin shifts in the presence of the ingredient—especially a foreign protein (unless it have the same iso-electric point)—or the salt. This has been fully discussed in publication[5] from our laboratory. Therefore, in discussing the character of glutenin it is most important to proceed with the same method of preparation and with the same method of determination of the iso-electric point. We have by our methods and with these precautions, isolated the glutenin and determined its iso-electric point.

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(1) Bailey C.H. & Peterson A.C., Journ. Ind. Eng. Chem., **13**, 916 (1921).
(2) Sharp P, F. & Gortner R. A., Minn. Agri. Exp. Sta., Tech. Bull. 19.
(3) Tague E., Journ. Amer. Chem. Soc., **47**, 418 (1925).
(4) Csonka E.A. & Jones D.B. , Journ.Biol. Chem., **73**, 320 (1927).
(5) The Preceding article of this memoirs.

B. Experimental Results

a. On the preparation of the glutenin.

The wheat kernel, bred at Hokkaido Agricultural Experimental Station and named Akagawa Aka 1., was ground into flour and freed from bran by means of a sieve. A certain amount of the flour was mixed with water and kneaded with the hands and then washed with running water until no starch particles came from the viscous mass, which is the crude gluten. This gluten was chopped and again washed with water to remove further the starch particles. Such a gluten chop from 2.5 Kg. of flour was mixed with 2 L. of 70% alcohol (by volume, etc) and stirred with a motor for about 12 hours.

The alcohol extract was removed and the residue was chopped and extracted with alcohol again in the manner described above. This procedure was repeated six times. The first extract was strongly yellowish in color, but the sixth extract was almost colorless. The dilution of the latter with water produced a faintly white turbidity. This showed that the sixth extract contained only a trace of gliadin and the greater part of the gliadin was extracted from the gluten after the successive six extractions. The extracted residue—the crude glutenin—was washed fully with water to remove any traces of alcohol and then mixed with 1.5 L. of water. To this mixture 1.5 L. of 0.05 n NaOH was added drop by drop and stirred to dissolve the glutenin. This solution was then filtered repeatedly by means of a Buchner funnel, fitted with purified filter-pulp, to remove any particles of starch. The filtrate thus obtained was clear and transparent.

It was dropped with 0.05 n. acetic acid under stirring until the glutenin precipitated out. This glutenin precipitate was treated again with 70% alcohol, and from it was extracted a certain amount of gliadin. This was repeated 2 or 3 times. In the former extraction the alcohol could not throughly catch out the gliadin from the gluten chops, even though the extraction was done successively six times. The latter treatment of the extraction—that is the washing with water to remove out the alcohol, dissolving with a dilute alkali solution, precipitation with acetic acid and then extracting with alcohol 2 or 3 times—was repeated twice more, until the residue (the glutenin) contained no trace of gliadin. Then the glutenin was dialysed to remove out the dialysable ingredients for about ten days, by our method.[1] The glutenin thus prepared was reserved in an ice-box

(1) Kondo K. & Hayashi T., This memoir, No. 2, Art. 1.

for further study. A part of this glutenin was treated with alcohol and ether successively and then in an incubator at 40°C freed from any trace of the latter. This anhydrous glutenin was a white voluminous powder and contained 17.17% of Kjeldahl nitrogen. The nitrogen content of the glutenin is reported as shown in the following table.

Table 1.

Author	Nitrogen content. %
König[1]	17.10–17.45
Osborne[2]	17.49
Woodman*	17.49
Halton*	17.42
Cross & Swain*	16.23–16.81
Hoffman & Gortner[3]	17.20
Csonka & Jones*	17.14 (α—glutenin) 16.06 (β—glutenin)
Kondo & Hayashi	17.17

b. Preparation of stock solution.

The glutenin, which was purified and dialysed in the manner described above, was voluminous and suspended. Hence, we made this glutenin in a compact precipitate by a centrifugal machine and then put a certain quantity of this precipitate with water into a 500 cc. measuring flask. After this, 125 cc. of 0.1 n NaOH, previously diluted with water into twice its volume, was dropped into the flask with continuous shaking and the flask was filled up with water at 15°C. Then the flask containing the protein was immersed in a water-bath at 18°C and occasionally shaken in order to dissolve the protein thoroughly. After 15 hours, we saw a yellowish brown viscous solution, which was then freed by means of the centrifugal machine from a certain portion of the protein at the bottom of the flask which could not be dissolved.

(1) König: Chemie d. Nahr. u. Genusm. II. 22.
(2) Osborne T.B. The Vegetable Proteins, **2.** 72.
(3) Hoffman W.F. & Gortner R.A., Cereal Chem. **4,** 221 (1927).
* Loc. cit.

After this procedure, the solution was filtered through a Buchner funnel to avoid the retention of any undissolved portion of the glutenin. The funnel was fitted in layers with purified filter-pulp, previously washed with water and with a 0.1 n NaOH solution. Of course, the first filtrate was removed from the solution. The filtrate is yellowish brown in color, and this was reserved in an ice-box in a colored bottle, which was surrounded by pieces of ice. This stock solution was 0.025 n in respect to the NaOH-concentration.

c. Determination of the iso-electric point.

Experiment 1. A certain volume of the stock solution was mixed with the acetic acid-acetate buffer solution of varying paH, and filled up with water to 50 cc. Care must be taken to keep the same concentration of acetate in each mixture. Then the amount of the precipitated glutenin and the paH value of the mixture respectively were determined. The precipitate was transferred on a filter paper (marked with Toyo F. P. No. 3) and washed with a solution of the same paH as that of the filtrate, and then kjeldahled. The amounts of the solutions, which were mixed to prepare the experimental solution, are tabulated in Table 2. a. The paH value of the experimental solution and the amount of the precipitated protein-nitrogen are summarized in Table 2. b.

Table 2. a

Experiment No.	Stock Solution	$NaCH_3COO$ 0.25 n	HCH_3COO 0.1 n	H_2O
	cc.	cc.	cc.	cc.
1	10	4	2.5	to 50
2	,,	,,	2.75	,,
3	,,	,,	3.25	,,
4	,,	,,	3.75	,,
5	,,	,,	5.25	,,
6	,,	,,	7.00	,,
7	,,	,,	8.00	,,
8	,,	,,	10.00	,,
9	,,	,,	12.00	,,
10	,,	,,	14.00	,,
11	,,	,,	16.00	,,
12	,,	,,	18.00	,,
13	,,	,,	20.00	,,
14	,,	,,	24.00	,,
15	,,	,,	28.00	,,

Table 2. b

Experiment No.	Total Protein-Nitrogen in Stock Solution	C_{NaCH_3COO}	C_{HCH_3COO}	Protein-Nitrogen precipitated (determined)	paH (determined)
	mg.	n	$10^4 \times n$	mg.	
1	32.95	0.025	0	25.51	6.265
2	,,	,,	5	25.56	6.140
3	,,	,,	15	26.41	5.823
4	,,	,,	25	26.80	5.708
5	,,	,,	55	**27.40**	**5.323**
6	,,	,,	90	**27.10**	**5.243**
7	,,	,,	110	26.80	5.107
8	,,	,,	150	26.80	4.937
9	,,	,,	190	26.60	4.882
10	,,	,,	230	25.81	4.747
11	,,	,,	270	25.96	4.674
12	,,	,,	310	25.76	4.607
13	,,	,,	350	25.32	4.498
14	,,	,,	430	25.02	4.423
15	,,	,,	510	24.72	4.219

According to the above results, our glutenin flocculates at a maximum in the acetic acid-acetate solution of paH 5.243–5.323, when the concentration of the acetate in the buffer solution is 0.025 n. And the percentage of the precipitated protein-nitrogen to the total nitrogen is 83.16. Therefore we may estimate that the apparent iso-electric point[1] of this gluteinin is paH 5.243–5.323.

Experiment 2. When an iso-electric point of a protein so difficult of solution as glutenin is determined by the above method, it is remarkably changeable according to the kind and concentration of the salts in the buffer solution.[2] This influence of the salt is comparatively weakened by using the diluted acetic acid-acetate mixture as a buffer solution. However, if the buffer solution be too diluted to avoid the

(1) Kondo K. & Hayashi T., This Memoir, No. 2 Art. 3. (1926).
(2) Kondo K. & Hayashi T., This memoir, No. 5. Art. 1. (1928).

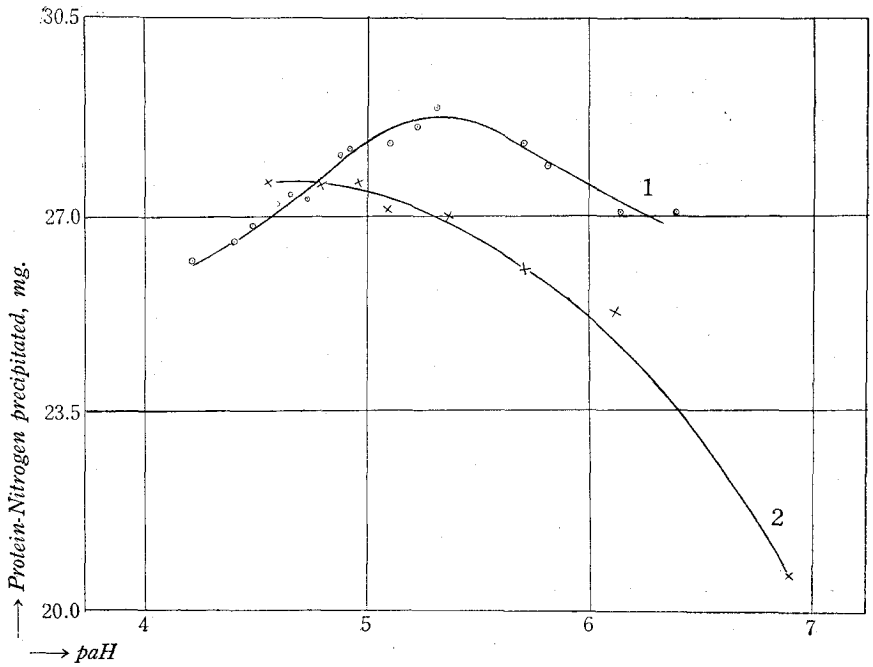
influence of the salt, there will be difficulty in the electrical determination of the paH-value. Hence, if we have an electrolyte, the presence of which does not shift the maximum flocculation point of the protein, then by the addition of such an electrolyte in a certain concentration to the buffer solution we may electrically find the iso-electric point of the protein in its precise value so far as it may be possible. Michaelis[1] has reported that the maximum flocculation point of a protein difficult of solution does not change in the presence of $NaCH_3COO$ or RbCl. After our experiments,[2] however, the presence of these salts also shifts the apparent iso-electric point of the protein. It is only that the range of shifting is small. If the concentration of the acetate be dilute and 0.01 n RbCl added to the buffer solution, the influence of the acetate will not only be weakened, but also the paH value of the solution can be determined more accurately. With this expectation, the maximum flocculation point of the glutenin was sought in a solution of 0.025 n $NaCH_3COO$, and 0.01 n RbCl. The results are summarized in Table 3 and Figure 1, but in the latter the results of experiment "1" are also shown.

Table 3.

Experiment No.	Total Protein-Nitrogen in Stock Solution	C_{RbCl}	C_{NaCH_3COO}	C_{HCH_3COO}	Protein-Nitrogen precipitated (determined)	paH (determined)
	mg.	n	n	$10^4 \times$ n	mg.	
1	32.95	0.01	0.025	* 10	0.77	7.733
2	,,	,,	,,	* 7	0.87	7.625
3	,,	,,	,,	* 5	18.57	6.879
4	,,	,,	,,	5	23.78	6.110
5	,,	,,	,,	25	24.57	5.700
6	,,	,,	,,	50	25.46	5.363
7	,,	,,	,,	110	25.66	5.106
8	,,	,,	,,	150	26.11	4.982
9	,,	,,	,,	190	**26.16**	**4.831**
10	,,	,,	,,	270	**26.16**	**4.569**

(1) Michaelis L. u. von Szent-Gyorgyi, Biochem. Z., **103**, 178 (1920).
(2) Kondo K. & Hayashi T., This Memoirs, No. 5. Art. 1. (1928)
* Figures, marked with *, show the concentration of NaOH instead of CH_3COOH.

Fig. 1.



As seen in the above results, it is clear that the addition of RbCl, even in diluted concentration, to the buffer solution shifts the maximum flocculation point to the acidic side. Moreover, the maximum amount of the precipitated protein counts only as 76.40% of the total protein, about 11% being reduced when compared with that in the case of the absence of RbCl.

Experiment 3. The theoretical iso-electric point of the ampholyte is represented by $C_H=\sqrt{\frac{Ka \times Kw}{Kb}}$, in which Ka and Kb represent the dissociation constants of the ampholyte as acid and as base respectively, and Kw represents the dissociation constant of watar. As we seen in the above equation, the hydrogen ion concentration at the iso-electric point of the ampholyte is independent of its concentration. Whether the apparent iso-electric point of the glutenin, which is determined by our method, is also independent of the glutenin concentration of the experimental solution or not, was examined with the following results.

Table 4.

Experiment No.	Total Protein-Nitrogen in Stock Solution	$CNaCH_3COO$	$CHCH_3COO$	Protein-Nitrogen precipitated (determined)	paH (determined)
	mg.	n	n	mg.	
1	49.43	0.025	0	37.63	6.263
2	,,	,,	0.0005	43.98	6.044
3	,,	,,	0.0020	45.71	5.727
4	,,	,,	0.0025	45.51	5.687
5	,,	,,	0.0050	**46.61**	**5.393**
6	,	,,	0.0055	**46.61**	**5.339**
7	,,	,,	0.0070	**46.61**	**5.229**
8	,,	,,	0.0085	**46.61**	**5.151**
9	,,	,,	0.0125	46.31	5.094
10	,,	,,	0.0215	46.16	4.772
11	,,	,,	0.0325	45.51	4.601
12	,.	,,	0.0375	44.63	4.550

Table 5.

Experiment No.	Total Protein-Nitrogen in Stock Solution	$CNaCH_3COO$	$CHCH_3COO$	Protein-Nitrogen precipitated (determined)	paH (determined)
	mg.	n	$10^4 \times n$	mg.	
1	16.48	0.025	0	10.61	6.393
2	,,	,,	5	11.80	6.127
3	,,	,,	15	12.25	5.837
4	,,	,,	25	12.40	5.703
5	,,	,,	40	12.54	5.522
6	,,	,,	55	**12.64**	**5.357**
7	,,	,,	75	**12.59**	**5.219**
8	,,	,,	95	12.54	5.126
9	,,	,,	115	12.44	5.024
10	,,	,,	175	12.35	4.886
11	,,	,,	215	12.40	4.782
12	,,	,,	295	12.15	4.663
13	,,	,,	335	12.05	4.499
14	,,	,,	375	11.30	4.468

The experimental results summarized in Table 4 and 5 are shown respectively in Figure 2 and 3.

Fig. 2.

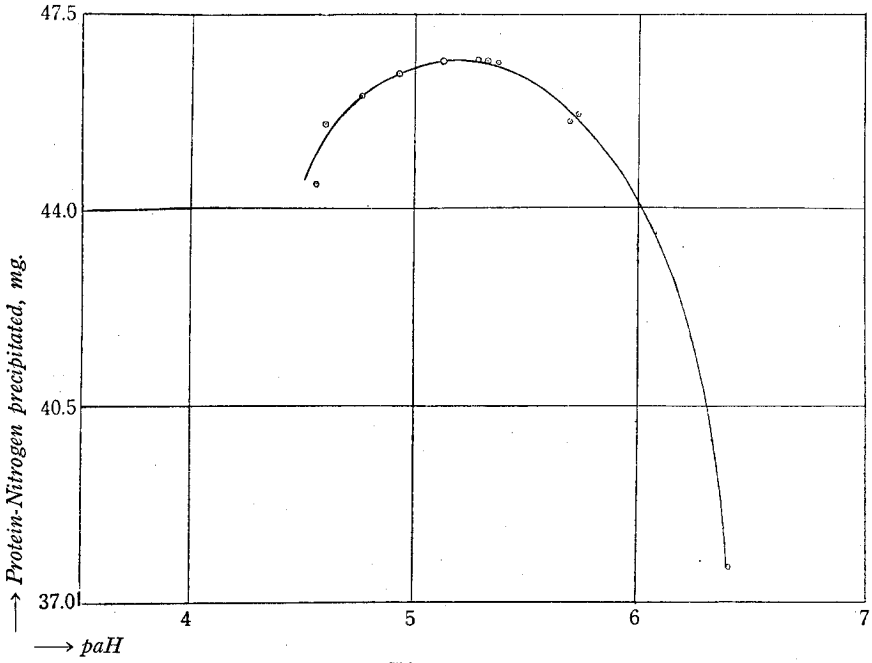
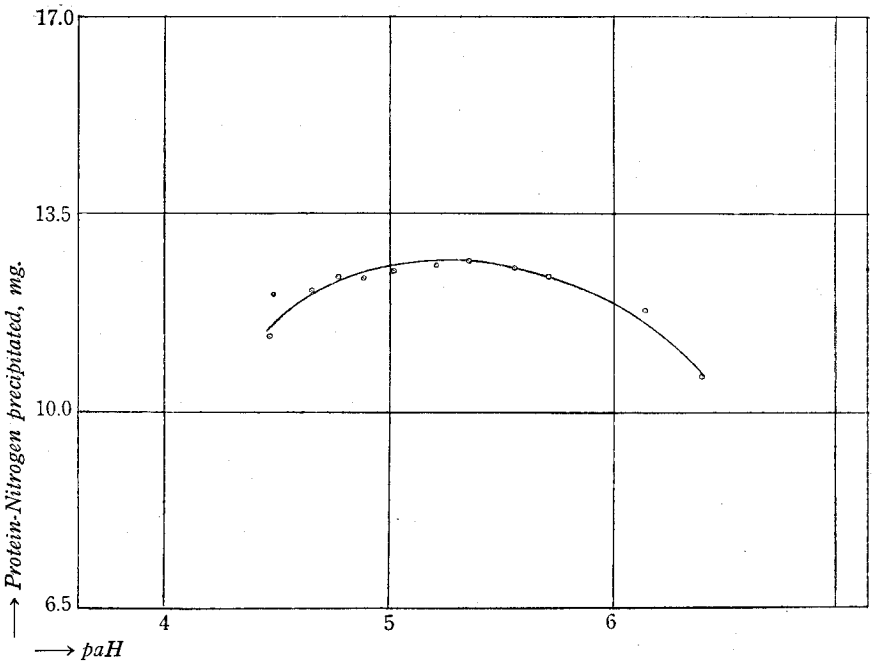

47.5
44.0
40.5
37.0
4
5
6
7
→ Protein-Nitrogen precipitated, mg.
→ paH

Fig. 3.


17.0
13.5
10.0
6.5
4
5
6
→ Protein-Nitrogen precipitated, mg.
→ paH

As shown in the above tables and Figure 2 and 3, an apparent iso-electric point of the glutenin falls on the paH value 5.151–5.393 and on the paH value 5.219–5.357 respectively, when the amount of total protein nitrogen in the experimental solution is about 46 mg and about 13 mg.

We have already ascertained after experiment "1" that the glutenin can flocculate at a maximum at the paH value 5.243–5.323, when the same buffer solution contains about 27 mg of the glutenin nitrogen. These apparent iso-electric points fall on approximately the same range. Therefore, we may assume that an apparent iso-electric point of the glutenin is independent of its concentration in the experimental solution. However, the more the concentration increases, the more the proportion of the precipitated protein-nitrogen to the total increases.

It counts respectively 76.70%, 83.16% and 94.29% according to the glutenin concentration of the experimental solutions. In every solution, the acetate concentration is equal, and hence, the amount of the dissolved protein in a certain volume of the solution of the same paH value must be always equal. Therefore, it is reasonable to assume that the more the glutenin concentration increases, the more the proportion of the precipitated glutenin to the total increases. However, the amounts of dissolved glutenin at the iso-electric point are not the same in the preceding 3 experiments, that is 2.84, 5.55 and 3.84 mg as nitrogen. This may be due to the differences in the final salt concentrations, which occur after protein-precipitation, and to experimental error.

Experiment 4. According to Blish and Sandstedt,[1] the physico-chemical properties of the glutenin may change, if it be dried at 60°–65°C. Even without heating, will not the treatment of glutenin with alcohol and ether alter its iso-electric point? To obtain an answer to this question, the following experiment was undertaken.

12 g of anhydrous glutenin were mixed with water in a 500 cc. measuring flask and then dissolved with diluted NaOH in the same manner as described in section "b". The NaOH-concentration of prepared solution was 0.025 n and it contained 37.19 mg of the glutenin nitrogen per 10 cc. With this solution the iso-electric point of the glutenin was determined again with the following results.

According to the above results, the anhydrous glutenin may flocculate at a maximum also in the range of paH value 5.164–5.310. In this range, the proportion of the precipitated glutenin to the total

(1) Blish and Sandstedt, Cereal Chem., **3**, 144 (1926).

Table 6.

Experiment No.	Total Protein Nitrogen in Stock Solution	C_{NaCH_3COO}	C_{HCH_3COO}	Protein-Nitrogen precipitated (determined)	paH (determined)
	mg.	n	$10^4 \times n$	mg.	
1	37.19	0.025	* 7	1.07	7.481
2	,,	,,	* 5	12.82	6.992
3	,,	,,	0	26.16	6.525
4	,,	,,	5	29.28	6.180
5	,,	,,	10	29.93	5.931
6	,,	,,	25	30.22	5.686
7	,,	,,	40	30.32	5.420
8	,,	,,	55	**30.72**	**5.310**
9	,,	,,	100	**30.52**	**5.164**
10	,,	,,	110	30.22	5.075
11	,,	,,	190	29.93	4.906
12	,,	,,	230	29 83	4.794
13	,,	,,	270	29.48	4.720

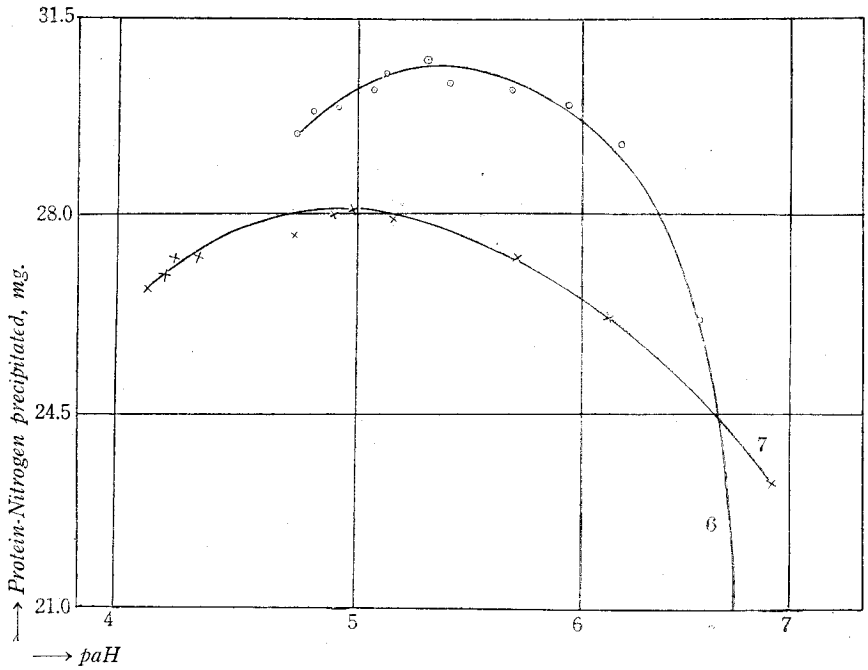
Figures, marked with *, represent the conc. of NaOH instead of HCH_3COO.

Table 7.

Experiment No.	Total Protein-Nitrogen in Stock Solution	C_{RbCl}	C_{NaCH_3COO}	C_{HCH_3COO}	Protein-Nitrogen precipitated (determined)	paH (determined)
	mg.	n	n	n	mg.	
1	37.19	0.01	0.025	* 0.0005	23.25	6.850
2	,,	,,	,,	0.0005	26.18	6.122
3	,,	,,	,,	0.0025	27.22	5.715
4	,,	,,	,,	0.010	27.91	5.156
5	,,	,,	,,	0.011	**28.11**	**4.986**
6	,,	,,	,,	0.015	**28.02**	**4.891**
7	,,	,,	,,	0.023	27.67	4.717
8	,,	,,	,,	0.035	27.25	4.288
9	,,	,,	,,	0.051	27.25	4.182
10	,,	,,	,,	0.059	26.92	4.166
11	,,	,,	,,	0.075	26.72	4.066

* It represents the conc. of NaOH instead of HCH_3COO.

Fig. 4.



counted as 82.6% and the amount of the dissolved glutenin in 50 cc. of the experimental solution was 6.47 mg as nitrogen. If we refer this results to previous results, we can easily understand that the iso-electric point of the glutenin does not shift, even after its dehydration with alcohol and ether. Next, the influence of RbCl on the iso-electric point of the glutenin, treated as above described, was also examined. The experimental results are summarized in Table 7 and Figure 4, and in the latter, the results, shown in Table 6, are also represented.

The above results show that in the presence of RbCl, even in its dilute concentration, the apparent iso-electric point of the glutenin may shift, and the amount of the precipitated protein decrease.

C. Discussion and Conclusion

The apparent iso-electric point of our glutenin may be the paH value 5.15–5.39. And this value may not change in any experiment, even though the protein concentration of the exeprimental solution be

changed, and only the constant salt concentration be held. This fact is in accordance with the theory of the iso-electric point.

However, we see that the more the protein concentration of the experimental solution increases, the more the proportion of the precipitated protein at its maximum flocculation point to the total increases. We have already discussed the fact that a protein so difficult of solution as casein or rice-glutelin, even in an iso-electrically reacting solution, can not completely take the form $\left[R^{-NH_2}_{-COOH}\right]$ under the presence of the salt in the solution. A part of the protein takes the form $\left[R^{-NH_2}_{-COOH}\right]$ or $\left[R^{-NH_2}_{+COOH}\right]$ and dissolves. This solubility may depend on the kind of the salt and its concentration. The glutenin will also behave like casein or rice-glutelin in its iso-electrically reacting solution.

In our experiments, the concentration of acetate in every experimental solution was equally 0.025 n.

Hence, the solubility of the protein at its maximum flocculation point must be constant and independent of the protein concentration. (The amount of the dissolved protein at its iso-electric point was not precisely equal in every experiment. However this difference must be due to experimental errors and the difference of the resulting salt concentration after the precipitation of the protein.) Therefore, according to this theory, it is reasonable to assume that the more the protein concentration increases, the more the proportion of the precipitated protein to the total protein at its maximum flocculation point increases.

Also the dehydrated glutenin as well as the hydrous glutenin show iso-electric behavior in the solution of the same hydrogen ion activity. This tells us that the glutenin can not denaturate after its dehydration with alcohol and ether. However, the addition of RbCl to the buffer solution shifts the apparent iso-electric point either of the hydrous glutenin or the dehydrated glutenin to the acidic side and moreover it reduces the amount of the precipitated protein. The cause of this is sought as already discussed in the preceding article, in the fact that the glutenin-ionizeing power of Rb^+ is less than that of Cl^-.

The iso-electric point of our glutenin shifts to the acidic side farther than those reported by other investigation. This is, of course, caused partly by the fact that our glutenin has not the same character as the glutenins, which were isolated by other experimenters. However, the cause may lie, in great part, in a difference in glutenin-purity and in the

modes for the determination of the iso-electric point of the protein. The old workers have considered that they could extract all of the gliadin from the gluten after repeated treatment of the gluten-grains with alcohol. However, this is not the case. As we have done, the glutenin may be freed from traces of gliadin, only when the crude glutenin-precipitate from its alkali solution is extracted again with alcohol. This crude glutenin is, of course, previously prepared after the full extraction of the gluten-grains with alcohol.

Herewith, it may be considered that the glutenins, which were prepared by a number of workers, might have contained a certain amount of gliadin. If a protein contain a certain amount of a foreign protein, its iso-electric point shifts unless the foreign protein have the same iso-electric point as the original protein.

Hence, if the glutenin, which was previously studied, contained a certain amount of gliadin, its iso-electric point was naturally different from that of our glutenin.

According to the previous experimental results, we may reasonably acknowledge that the glutenin differs in its character from one variety of wheat to another. However, to prove this assumption we must first prepare the glutenin from various flours by our method and then further study its character. This study, with such precautions as are described above, is being continued in our laboratory.

D. Summary

The results of the experiments discussed above are summarized as follows:—

(1) We prepared the purified glutenin with special care from the wheat kernel, which had been bred at Hokkaido Agricultural Expt. Station and called Akagawa Aka 1. The nitrogen content of this glutenin is 17.17% after the Kjeldahl method.

(2) The apparent iso-electric point of our glutenin falls on the paH value 5.15–5.39.

(3) This iso-electric point is independent of the glutenin concentration of the experimental solution. However, the higher the protein concentration of the experimental solution increases, the more the proportion of the precipitated protein to the total at its maximum flocculation point increases. This is due to the fact that the solubility of the protein at its maximum flocculation point is constant in a solution of a certain concentration of the salt. This is explained by our theory.

(4) The glutenin does not denaturate even after dehydration with alcohol and ether.

(5) The addition of RbCl to the buffer solution shifts the iso-electric point of the glutenin to the acidic side.

(6) The character of glutenin from various flours must be studied after it is prepared by our method.

(*April 18, 1928*)