PHYSICO-CHEMICAL STUDIES OF STRONG AND WEAK FLOURS. II. THE IMBIBITIONAL PROPERTIES OF THE GLUTENS FROM STRONG AND WEAK FLOURS*

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Introduction

In the first paper of this series Gortner and Doherty¹ undertook a study of certain physico-chemical properties of the glutens from strong and weak flours, inasmuch as the purely chemical method of attack by numerous other workers had not led to any consistent results. To be sure, Upson and Calvin^{2, 3} had previously investigated the colloidal swelling of gluten and had concluded that strong and weak glutens were determined by the effect of salts and acids upon the gluten colloids. Their reasoning was from analogy, however, rather than from actual experimentation with the two types of glutens. As a result of their own investigations Gortner and Doherty concluded that the postulations of Upson and Calvin did not hold, that acids and salts, while they influence the gluten markedly, are not the primary cause of weakness or strength but that there is an inherent difference in the colloidal properties of the gluten gel as laid down in the endosperm of the wheat berry, and that the colloidal properties of the glutens

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¹ Gortner, R. A., and Doherty, E. H.: Hydration capacity of gluten from "strong" and "weak" flours. Jour. Agr. Res., 13, 389-418, Fig. 17, May 20 (1918).

² Upson, F. W., and Calvin, J. W.: On the colloidal swelling of wheat gluten. Jour. Am. Chem. Soc., 37, 1295-1304, Fig. 3, pl. 2 (1915).

³ Upson, F. W., and Calvin, J. W.: The colloidal swelling of wheat gluten in relation to milling and baking. Nebr. Agr. Exp. Sta. Res. Bul., No. 8, Fig. 5, June (1916).

from strong and weak flours are not identical, even at the isoelectric point, and would not be identical even if the flours had originally had the same acid and salt content.

The problem therefore is a deeper one than merely a study of the action of salts or acids on the physical properties of a colloid. It is a theorem in colloid chemistry that a colloidal system has a "memory," that the colloidal behavior of an emulsoid sol or an emulsoid gel is dependent not alone upon its present environment but upon its past history, and it would seem that the past history of the gluten while it is being deposited in the endosperm of the wheat berry is the determining factor of a strong or of a weak flour.⁴

The historical treatment of the strong and weak flour question was considered in the first paper of this series and it is unnecessary to repeat it here. Such papers as bear upon the subject under consideration will be considered from time to time as later papers are prepared for publication.

Since the appearance of the paper by Gortner and Doherty, Ostwald⁵ and Lüers⁶ and Lüers and Ostwald^{7,8,9} have published a series of papers on the colloid chemistry of bread. It is not our intention to discuss their data in the present paper,

⁴ In our work on strong and weak flours we are coming to recognize at least three classes of weak flours, i. e., (1) weakness due to an adequate *quantity* of gluten but an *inferior quality*; (2) weakness due to an *inadequate quantity* of a good quality of gluten and (3) weakness due to factors influencing yeast activity, such as inadequate diastatic activity, acidity and amount of proteolytic enzyme, etc. The present paper and the definition above refer only to flours whose weakness is of the first type.

⁵ Ostwald, W. O.: Beiträge zur Kolloidchemie des Brotes I. Über kolloidchemische Probleme bei der Brotbereitung. Koll Zeit., **25**, 26-45, Fig. 1 (1919).

⁶ Lüers, H.: Beiträge zur Kolloidchemie des Brotes III. Kolloidchemische Studien am Roggen- und Weizen-gliadin mit besonderer Berücksichtigung des Kleber- und Backfähigkeitsproblems. Ibid., **25**, 177-196; 230-240, Fig. 5 (1919).

⁷ Lüers and Ostwald, W. O.: Beiträge zur Kolloidchemie des Brotes II. Zur Viskosimetrie der Mehle. Ibid., **25**, 82-90; and 116-136, Fig. 16 (1919).

⁸ Lüers, H., and Ostwald, W. O.: Beiträge zur Kolloidchemie des Brotes. IV. Zur Kenntnis von Mehlen schlechter Backfähigkeit. Ibid., **26**, 66-67 (1920).

⁹ Lüers, H., and Ostwald, W. O.: Beiträge zur Kolloidchemie des Brotes. V. Die Kolloide Quellung des Weizenklebers. Ibid., **27**, 34-37 (1920).

inasmuch as such a discussion properly belongs in the third paper of this series and will shortly be presented there.

Lüers and Ostwald⁹ in a fifth paper call attention to the work of Upson and Calvin and state that their own results are in substantial agreement. They add that there may be a difference in the quality of the gluten from different flours, but that the quality is profoundly influenced by salts and acids present in the flour. These workers make no mention of the work by Gortner and Doherty and they, like Upson and Calvin, reason from analogy rather than from actual experiments made upon both strong and weak flours. While in many instances it is possible to reach valid conclusions by such a method, it is a rather dangerous procedure when the material in question is an emulsoid gel. The same criticism applies to certain of the conclusions of Henderson and his co-workers^{10,11,12,13,14} in so far as their statements deal with flours of different baking strength where the *quality* of the gluten is the determining factor. For example, Cohn and Henderson¹⁰ state, "The 'body' of the dough is supplied by the wheat gluten alone. The degree to which the dough can be distended therefore depends upon the amount and the hydration of the gluten." Whether or not this statement is intended to apply to flours of different baking strength or only to war breads where wheat flour is diluted with other cereal flours cannot be determined from the context.

It is impossible to determine from the data given in the papers of Henderson, et al. exactly what type of flours they

¹⁰ Cohn, E. J., and Henderson, L. J.: The physical chemistry of bread making. *Science*, **48**, 501-505 (1918).

¹¹ Cohn, E. J., Wolbach, S. B., Henderson L. J., and Cathcart, P. H.: On the control of rope in bread. *Jour. Gen. Physiol.*, **1**, 221-230 (1918).

¹² Cohn, E. J., Cathcart, P. H., and Henderson, L. J.: The measurement of the acidity of bread. *Jour. Biol. Chem.*, **36**, 581-586, Fig. 1 (1918).

¹³ Henderson, L. J., Fenn, W. O., and Cohn, E. J.: Influence of electrolytes upon the viscosity of dough. *Jour. Gen. Physiol.*, **1**, 387-397, Fig. 5 (1919).

¹⁴ Henderson, L. J., Cohn, E. J., Cathcart, P. H., Wachman, J. D., and Fenn, W. O.: A study of the action of acid and alkali on gluten. *Jour. Gen. Physiol.*, **1**, 459-472, Fig. 1 (1919).

worked with, for baking tests and flour analyses are lacking. However, from the form of the viscosity curves (13, Fig. 2, p. 391) it appears probable that flour "A" is markedly superior in gluten quality to the other three flours. At any rate their curves show that while viscosity may be *altered* by a change in hydrogen ion concentration, the initial viscosity of the dough is not determined *solely* by this factor. Of course it is possible that all of the flours possessed approximately the same *quality* of gluten, but that flour "A" had a greater *quantity*, in which case a higher initial viscosity would result. Unfortunately no nitrogen determinations are available, nor is the source or grade of the samples noted.

Martin¹⁵ has recently carried out an investigation of some of the properties of wheat flour which he thought might be correlated with strength. He finds for the flours which he investigated that "flours with high gas-retaining capacities and high bakers' marks have been shown to be those in which the 'amended gliadin' figure is high." "For flours having a satisfactory gas-producing capacity, bakers' marks, gas-retaining capacity, and 'amended gliadin' content are closely related, and it is considered that the estimation of either of the latter together with the determination of the gas-producing capacity will indicate the 'strength' of the flour."

The amended gliadin figures as used by Martin were determined by subtracting from the percent of protein ($N \times 5.7$) extracted from a sample of flour in a Soxhlet extractor by means of 50% alcohol, the percent of protein ($N \times 5.7$) extracted from 25 grams of flour by 250 cc of water at 24–25°, the time of extraction being three hours.

It would be interesting to know whether or not the proteolytic enzyme activity *varied* with the different flours which Martin used. In one of the flours which he worked with he showed that there was appreciable proteolytic activity. He also pointed out that the diastatic activity varied with the

¹⁵ Martin, F. J.: Properties affecting strength in wheaten flour. Jour. Soc. Chem. Ind., 39, 246–251 (1920).

flours used. If proteolytic activity varied this would influence the amount of water-soluble nitrogen and accordingly his "amended gliadin" values.

Experimental

The Problem.—Gortner and Doherty (p. 417) sum up their investigation on the hydration capacity of gluten as follows: "The difference between a strong and weak gluten is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoids, and that of a colloidal gel in which these properties are much less marked. It is suggested that such differences may be due to the size of the gluten particles and that at least a part of the particles comprising the weak gluten may lie nearer the boundary between the colloidal and crystalloidal states of matter than is the case with the stronger glutes."

The investigations reported in this paper and those which will follow were undertaken in order to put the above hypothesis to a test, and to see if other physico-chemical properties of the glutes from strong and weak flours could be correlated with flour strength.

Unfortunately none of the flours with which Gortner and Doherty worked were available for the present series of investigations. Consequently it became necessary to repeat at least a part of their work using gluten from new samples of flours in order that the comparative data for the present set of flours would be complete. The present paper is devoted to a consideration of the hydration or imbibitional properties of the new samples of flours. The following physico-chemical properties of the glutes are considered:

1. Imbibition in the presence of different strengths of lactic and hydrochloric acids.
2. Imbibition in the presence of different strengths of the following alkalis: potassium hydroxide, sodium hydroxide, calcium hydroxide, barium hydroxide and ammonium hydroxide.
3. The effect of M/200 sodium sulfate on the rate of imbibition in solutions of potassium and calcium hydroxide.

4. The optimum hydrogen ion concentration for the swelling of discs in the following acids: lactic, acetic, orthophosphoric, hydrochloric, and oxalic.

The Material used.—The strong flour used, B-780, was milled from northern spring wheat and was an especially high grade patent flour prepared for a select trade. B-781 is a first clear flour made in the same mill as B-780. The weak flours used were B-782, a patent milled from a soft western wheat grown around Waitsburg, Washington, and B-783, a patent milled from soft club wheat grown at Genessee, Idaho.

Flour Analyses and Baking Tests.—Table I shows certain of the analytical values usually determined on flours and also records the conventional baking test values.

TABLE I
Flour Analyses and Baking Tests

Lab. no. of flour	Ash content of flour, %	Crude protein in flour (N $\times$ 5.7) dry basis, %	Dry matter in wet gluten; ave. of 5 determinations, %	Water added per 100 g of flour to make dough, cc	Vol. of fermented dough given by 150 g of flour, cc	Volume of loaf, cc	Color score of loaf B-780 as 100	Texture of loaf B-780 as 100
B-780	0.44	12.13	32.51	57.9	733	1639	100	100
B-781	0.90	14.25	37.55	59.7	820	1580	98	101
B-782	0.49	9.58	36.63	59.7	900	1430	100	101
B-783	0.53	12.03	35.25	57.4	715	1530	100	98

The baking values given for B-780 are the average of three bakings, and those for B-783 are the average of two independent bakings.

It was thought advisable to repeat the bakings with flours B-780 and B-783 and also inasmuch as a low volume might be due to deficiency of soluble carbohydrates to investigate the effect of diastase. Jessen-Hansen¹⁶ has pointed out that the optimum hydrogen ion concentration for baking is around pH value of 5.0 and that the volume of the loaf may be in-

¹⁶ Jessen-Hansen, H.: *Etudes sur la farine de froment. I. Influence de la concentration en ions hydrogène sur la valeur boulangère de la farine. Comptes-rendus des Travaux Lab. de Carlsberg*, 10, p. 170-206, Fig. 4 (1911).

creased by the addition of acid if the pH without acid would be greater than pH 5.0. In order to partially test this conclusion and to see what effect the addition of acid would have on the relative volume of the loaves produced with the two flours, 2 cc of normal lactic acid per 475 grams of flour were added to one set. It is regretted that the supply of flours did not permit further tests along this line, but the tests tend to show the superiority of flour B-780. The baker noted that flour B-780 indicated its superiority in the handling of the dough; it was more firm and elastic and did not break in the kneading process. The method and baking formula as described by Bailey¹⁷ was used. The amount of an especially active diastase preparation necessary to bolster up flours low in diastase had been previously determined. The hydrogen ion concentration of the baked loaf was determined by the potentiometric method 12 hours after baking. For this determination 8 grams from the center of the loaf were mixed with 20 cc of water and 15 cc of the mixture placed in a large Bailey¹⁸ electrode which handles such mixtures easily. The results are given in Table II. The pH values were checked by duplicate determinations which agreed within 1 millivolt.

TABLE II

Baking Test of Flours B-780 and B-783 giving the Volume of Loaf and the Hydrogen Ion Concentration of the Baked Bread on Normal Doughs, Doughs to Which Lactic Acid has been added, and Doughs to which Diastase has been added

	B-780		B-783	
	Volume of loaf, cc.	pH of baked bread	Volume of loaf, cc.	pH of baked bread
Normal control loaf	1630	5.47	1520	5.49
Loaf to which lactic acid has been added	1570	5.22	1360	5.36
Loaf to which diastase has been added	1690	5.40	1220	5.52

¹⁷ Bailey, C. H: A method for the determination of the strength and baking qualities of wheat flour. *Jour. Ind. Eng. Chem.*, **8**, p. 53-57, Fig. 1 (1916).

¹⁸ Bailey, C. H: A simple hydrogen electrode. *Jour. Am. Chem. Soc.*, **42**, 45-48, Fig. 2 (1920).

In order to ascertain whether or not the amended gliadin figure of Martin¹⁵ differed in the two flours worked with, the protein fractions were determined by several methods. The first and third determinations were carried out essentially as described by the Association of Official Agricultural Chemists.¹⁹

Potassium Sulfate-Soluble Protein.—12 grams of the flour were weighed into a 500 cc flask and exactly 200 cc of a 5% potassium sulfate solution, measured at 25° and then cooled to 8–10°, were added; the mixture was shaken at 30 minute intervals for 3 hours and filtered. All of this process was carried out at a temperature of from 8–10°. The filtrate was brought to the temperature of 25° and two 50 cc aliquots taken with a pipette calibrated at 25°. Nitrogen in these aliquots was determined by the Kjeldahl method. The protein was calculated from the amount of nitrogen by the use of the factor 5.7.

Water-Soluble Protein.—20 grams of flour were treated with exactly 200 cc of water and shaken at frequent intervals for 40 minutes and then filtered, the whole process taking place at from 8–10° C. Two 50 cc aliquots were taken and protein determined as above.

Alcohol-Soluble Protein.—4 grams of flour were treated with exactly 200 cc of 70% alcohol by volume, and shaken at convenient intervals during 24 hours. The material was filtered and the protein content determined as above.

Water-Soluble Protein at the End of 3 Hours at 25°.—For this determination 20 grams of flour were treated with exactly 200 cc of distilled water and allowed to stand for 3 hours with frequent shaking in a water bath at 25° ± 0.01°. At the end of this time the material was filtered and nitrogen determined on aliquots of the filtrate.

Potassium Sulfate-Soluble Protein by Centrifuge Method.—About 42 cc of a 5% potassium sulfate solution were shaken with 3 grams of flour; the suspension was then centrifuged

¹⁹ "Methods of Analysis." Association of Official Agricultural Chemists, Washington, 1920, pp. 167–168.

and the clear supernatant liquid decanted into a Kjeldahl flask. The residue remaining was then treated with a fresh portion of potassium sulfate solution and the process repeated. This extraction was continued until six separate portions of potassium sulfate solution had been used or a total of about 250 cc. Nitrogen was determined on the material in the Kjeldahl flask and from this the protein content was calculated by the factor 5.7.

Alcohol-Soluble Protein in the Residue after Extraction with Potassium Sulfate.—The residue from the potassium sulfate extraction above was treated with 250 cc of 70% alcohol in six separate portions, the procedure being the same as for the above potassium sulfate extraction. Protein was determined on the total alcohol extract by the Kjeldahl method as described above.

Residue from the Potassium Sulfate and Alcohol Extractions.—The residue remaining after extraction first by the six portions of potassium sulfate and subsequently by six portions of 70% alcohol was placed in a Kjeldahl flask and protein determined as described above.

Total Protein of Flour.—The total protein of the flour was determined on 1 gram samples and calculated on the basis of $N \times 5.7$.

These determinations were all carried out in duplicate. The average of the different determinations is given in Table III.

These results show by whatever method the amended gliadin figure is calculated that flour B-783 has the higher figure and should according to Martin have the higher baking strength. The reverse is actually the case.

The Moist Gluten.—The gluten was easily obtained from sample B-780 and was a firm elastic mass that could be separated from the starch very rapidly. More difficulty was evidenced in collecting the gluten from B-781; it was rather intermediate between B-780 and B-782 and B-783. The glutens from these last two flours could be collected only with extreme difficulty. They were whiter, it was harder

TABLE III

The Various Protein Fractions of Flours B-780 and B-783 (All Results calculated on the Dry Basis). Protein obtained by $N \times 5.7$ in Each Case

	Flour B-780, %	Flour B-783, %
Potassium sulfate-soluble proteins, A. O. A. C. method (modified)	1.63	1.67
Water-soluble protein at the end of 40 minutes at 10° C.	1.59	1.24
Alcohol-soluble protein, A. O. A. C. method	6.60	7.31
Water-soluble protein at the end of 3 hours at 25° C.	1.85	1.67
Potassium sulfate-soluble protein by the centrifuge method	3.09	2.94
Alcohol-soluble protein in residue after extraction with potassium sulfate by the centrifuge method	5.73	6.41
Residue from the above potassium sulfate and alcohol extraction	3.32	2.80
Total protein from the sum of the last three determinations	12.14	12.03
Total protein as determined on the original flour	12.13	12.15

to separate them from the starch, and they were not firm or elastic. It is particularly interesting to compare the data for B-780 and B-783, the "strongest" and the "weakest" samples studied. The ash values are not widely different and are indicative of a "patent" and a "straight" flour. The crude protein values are almost identical so that, in this instance at least, the "weakness" of B-783 does not lie in a lower gluten content. The marked contrast in the baking tests shows that in these contrasting samples we have excellent material for testing as to whether or not the differences observed in the baking tests are paralleled by differences in the physico-chemical behavior of the gluteins.

The Preparation of Dried Glutens.—It is obvious that if the gluten could be washed from a large quantity of flour and then dried, this dried material would be a much more satisfactory type of material to use in physico-chemical work than would the moist gluten because each day's material would then be exactly comparable. Using dried material

would also save a considerable amount of time. Therefore the attempt was made to prepare considerable quantities of the dried glutens washed from the four flours.

Wheat gluten is an emulsoid colloid and it is well known that emulsoid colloids will not withstand the effect of high temperature. Consequently the drying must necessarily be carried out at a temperature that would not be at all unfavorable to bacterial and enzyme action. The first attempts to dry the gluten were made in a vacuum oven at a temperature of about 30° but the moist gluten retains the water rather tenaciously and the drying could not be completed at this temperature before the gluten began to decompose. It was finally found necessary to dry at a temperature of 45–50°. The method finally adopted for preparing the dried gluten was as follows:

About 3 kilos of flour were made into a stiff dough with distilled water and allowed to stand under distilled water for an hour. The material was then placed in an electrically driven dough-mixing machine. Distilled water under considerable pressure was run continuously into the mixer at a rather rapid rate. At the end of about 7 minutes all of the starch appeared to have been removed, the liquid from then on remaining only slightly turbid; the treatment was continued for 13 minutes longer, the water by this time had begun to froth slightly. The moist gluten was then pressed out between glass plates into sheets approximately 3 mm thick and was cut into small squares of about 1 sq cm area weighing less than 0.5 gram. Glass plates were used for shelves in the vacuum oven and these little squares were placed on the glass plates at least 1 centimeter apart. The material was then placed in the vacuum oven and dried at a temperature of 45–50° under a pressure of 30–40 mm. It was possible to dry the material to a crisp in less than 18 hours by this method, the drying was, however, continued for a total of 48 hours. This material showed no evidence of decomposition that could be detected by odor. The dried material was then placed in a ball mill and ground to a fine powder. The powder so ob-

tained was quite hygroscopic and was triboelectric. The dried material was preserved in tightly stoppered glass bottles. Inasmuch as opportunity for the absorption of moisture was afforded during the standing, etc., moisture determinations were made on the dried ground material by heating 18 hours in vacuo at 100°. The percentage of crude protein was likewise determined by multiplying the total nitrogen by the factor 5.70. The percentages of moisture and crude protein are recorded in Table IV. The values given are averages of closely agreeing duplicate determinations.

TABLE IV
Moisture and Crude Protein in the Dried Glutens

Lab. no.	Moisture, %	Crude protein in dried gluten, %
B-780	6.29	79.42
B-781	5.89	75.28
B-782	7.30	56.27
B-783	7.13	65.86

1. Imbibition in the Presence of Different Strengths of Hydrochloric and Lactic Acids

(a) *Relative Imbibitional Capacity of the Glutens before Drying.*—Gortner and Doherty¹ studied the effect of lactic, acetic, boric, phosphoric, hydrochloric and oxalic acids in various concentrations on the imbibition of glutens. From their data it is evident that two types of results may be expected, i. e., a strong acid will produce marked imbibition at the lower acid concentration followed by marked dehydration effects at higher acid concentrations. On the other hand, a weak acid produces marked imbibition throughout the entire range of concentration tested or from N/500 to N/2 concentration.

We have felt that it would not be profitable to repeat all of the work of Gortner and Doherty using the new flours, and have accordingly selected hydrochloric acid as typifying one type of imbibition phenomena and lactic acid as typifying the other. The *modus operandi* was the same as in the earlier paper (Gortner and Doherty I). The discs of gluten were al-

lowed to remain in the acid solution for exactly 50 minutes, then drained for 7 minutes and weighed.

In all of the work the value reported is the average of 5 individual determinations. An example of the determination on the basis of the gain in weight of the individual discs per gram of moist gluten is given in Table V. The gluten used was B-780. The temperature of all swelling experiments was $20^{\circ} \pm 2^{\circ}$. It will be noted that while some of the individual determinations vary, as a whole the agreement is good, and we believe that the average value is a reliable evidence of the degree of hydration, under the conditions of the experiment.

TABLE V

Quantity of Water imbibed (Grams of Water per Gram of Moist Gluten) from Individual Discs of Gluten B-780 in Different Concentrations of Hydrochloric Acid. Imbibition Period 50 Minutes

Acid concentration	N/2	N/5	N/10	N/25	N/50	N/100	N/200	N/500
Water imbibed	-0.024	0.055	0.230	0.696	1.050	0.863	0.846	0.747
per g of	-0.058	0.066	0.170	0.730	0.665	0.895	0.855	0.642
moist	-0.031	0.075	0.250	0.635	1.017	0.808	0.812	0.850
gluten	-0.028	0.050	0.200	0.650	0.960	1.085	0.774	0.574
	-0.032	0.026	0.275	0.720	0.921	1.040	0.577	0.822
Average	-0.035	0.054	0.225	0.686	0.923	0.938	0.773	0.727

TABLE VI

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Various Glutens (before drying) in Different Concentrations of Hydrochloric Acid. Imbibition Period 50 Minutes. (Average of 5 Determinations)

Concentration of acid	B-780	B-781	B-782	B-783
N/2	-0.035	-0.024	0.028	-0.035
N/5	0.054	0.061	0.061	0.033
N/10	0.225	0.150	0.155	0.151
N/25	0.686	0.400	0.374	0.367
N/50	0.923	0.553	0.514	0.453
N/100	0.938	0.633	0.422	0.477
N/200	0.773	0.517	0.459	0.478
N/500	0.727	0.296	0.428	0.412

Table VI shows average imbibitional values for all four glutens. The data of Table VI are shown in the form of curves in Fig. 1.

Fig. 1

The imbibition curves for the various glutens in different concentrations of hydrochloric acid (before drying, imbibition period 50 minutes).

There is a distinct difference in the swelling capacity in hydrochloric acid between the strong gluten, B-780, and the other three.

TABLE VII

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Various Glutens (before Drying) in Different Concentrations of Lactic Acid. Imbibition Period 50 Minutes. (Average of 5 Determinations)

Concentration of acid	B-780	B-781	B-782	B-783
N/2	1.328	0.669	0.546	0.406
N/5	1.395	0.701	0.687	0.476
N/10	1.518	0.816	0.627	0.528
N/25	1.552	0.603	0.580	0.572
N/50	1.417	0.642	0.611	0.598
N/100	1.080	0.367	0.487	0.521
N/200	1.231	0.421	0.457	0.460
N/500	0.787	0.232	0.467	0.497

Table VII and Fig. 2 show the average imbibitional values for the four glutens (before drying) in different concentrations of lactic acid.

Fig. 2

The imbibition curves for the various glutes in different concentrations of lactic acid (before drying, imbibition period 50 minutes).

It will be observed that there is a wide difference in the imbibitional capacity of glutes B-780 and B-781 and there is also a marked difference between glutes B-781, B-782 and B-783. The difference between these glutes is more marked than that found by Gortner and Doherty to exist between their "strong" and "weak" glutes, the "strong" flour B-780 being stronger than the one worked with by them, and the "weak" flours being weaker, as shown by imbibitional experiments.

(b) *Relative Imbibitional Capacities of the Glutes after drying.*—The dried gluten, prepared as noted above, was converted into moist gluten discs in order that the imbibitional values might be comparable with those determined on the freshly washed out gluten. Twenty grams of the dried gluten

were mixed with just enough water to make a rather dry dough. This dough was allowed to stand under distilled water for one hour. The gluten so obtained was much more tough and rubberlike than the original gluten before drying. In order to obtain sheets of uniform thickness it was necessary to press it out between the glass plates as thin as possible and weights had to be placed on the glass plates in order to keep the gluten from springing back into a mass. After remaining under pressure for some time additional pressure was applied and the sheet could then be pressed a little thinner. This process was repeated until sheets about 3 mm thick were obtained. Discs were then cut which were not placed in water for a preliminary period but were placed directly into the various solutions. Two sets of experiments were made. The first set is directly comparable with the moist gluten imbibitional experiments noted above where an imbibitional period of 50 minutes was used. In the second set the imbibitional period was reduced to 25 minutes inasmuch, as will appear later, it was desirable to compare imbibition in alkali with imbibition in acid and a fifty minute imbibitional period could not be used in the alkaline solutions. In addition the dilutions of the 25 minute set are not identical with the preceding acid sets, but correspond with the dilutions which are used later in this paper for the various alkalies.

Fifty Minute Imbibitional Experiments.—In view of the fact that hydrochloric acid did not appear to differentiate the glutens nearly so well as did lactic acid, no experiments were made in which the imbibitional capacity of the various dried glutens were tested in the various concentrations of hydrochloric acid. Table VIII and Fig. 3 show the imbibitional data for the different dried glutens, prepared as noted above, in different concentrations of lactic acid.

Twenty-Five Minute Imbibitional Experiments.—In order to directly compare the action of acids and alkalies as noted above the swelling in acids was carried out for 25 minute periods in the same concentrations that were used for the al-

TABLE VIII

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Various Glutens (after drying and milling the Gluten) in Different Concentrations of Lactic Acid. Imbibition Period 50 Minutes. (Average of 5 Determinations)

Concentration of acid	B-780	B-781	B-782	B-783
N/2	0.801	0.731	0.469	0.690
N/5	0.972	0.625	0.508	0.690
N/10	1.015	0.628	0.501	0.764
N/25	0.887	0.625	0.407	0.684
N/50	0.920	0.558	0.409	0.592
N/100	0.791	0.435	0.383	0.536
N/200	0.647	0.365	0.381	0.486
N/500	0.520	0.453	0.424	0.354
Water	0.039	0.063	0.026	0.042

Fig. 3

The imbibition curves for the various glutens in different concentrations of lactic acid (after drying, imbibition period). The top curve is B-780, the second B-783, the third B-781, and the lowest B-782.

kalies as noted later in this paper. The data obtained with lactic acid are given in Table IX and graphically in Fig. 4 and those obtained with hydrochloric acid are given in Table X and are also expressed graphically in Fig. 4.

Fig. 4

The imbibition curves for the various glutens in the same concentrations of hydrochloric and lactic acids as used in the case of imbibition in alkalis (before and after drying, imbibition period 25 minutes).

TABLE IX

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Various Glutens (before and after drying) in Different Concentrations of Lactic Acid. Imbibition Period 25 Minutes. (Average of 5 Determinations)

Concentration of acid	B-780		B-781	B-782	B-783	
	Before drying	After drying	After drying	Before drying	Before drying	After drying
N/100	0.509	0.411	0.195	0.329	0.259	0.427
N/200	0.458	0.311	0.127	0.234	0.255	0.231
N/500	0.332	0.253	0.071	0.184	0.207	0.142
N/1000	0.277	0.196	0.043	0.135	0.149	0.103
Water	-0.007	0.020	0.024	-0.031	-0.014	0.044

TABLE X

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Various Glutens (before and after drying) in Different Concentrations of Hydrochloric Acid Imbibition Period 25 Minutes. (Average of 5 Determinations)

Concentration of acid	B-780		B-782	B-783
	Before drying	After drying	Before drying	Before drying
N/100	0.687	0.378	0.375	0.247
N/200	0.501	0.227	0.317	0.222
N/500	0.346	0.129	0.223	0.185
N/1000	0.259	0.091	0.198	0.176
Water	-0.007	0.020	-0.031	-0.014

It is of interest to note that in the 50 minute experiments B-780 has decreased in its imbibing capacity in lactic acid to a very appreciable extent, that B-781 has decreased somewhat but the other two glutens have not changed to so marked a degree. This appears to indicate that the glutens are tending to become alike. The change is if anything even more marked in the 25 minute sets where an enormous decrease in imbibing capacity is noted for the dried gluten of B-780. In all of the glutens the concentration of acid necessary to produce maximum imbibition has likewise increased. Thus with B-780 maximum imbibition is attained in the undried gluten in N/25 lactic acid while in the dried gluten the corresponding point is in N/10 acid. Similarly for B-783 the values are N/50 and N/10, respectively. It has been our experience that weak glutens reach their point of maximum imbibition only in a higher concentration of acids than do strong glutens, and in the present instance the drying process appears to have produced a "weak" gluten as measured by rate and extent of imbibition. We know that alternate hydration and dehydration, or freezing and thawing, are some of the more common methods for coagulating or deflocculating colloids, and the present observation is a direct confirmation of the hypothesis set forth by Gortner and Doherty that a strong gluten is strong because of more marked col-

loidal properties, a part of which colloidal properties are lost when the gluten gel is dried, remilled and again made into a gel.

2. Imbibition in the Presence of Different Strengths of the Following Alkalies: Potassium Hydroxide, Sodium Hydroxide, Calcium Hydroxide, Barium Hydroxide, and Ammonium Hydroxide

(a) *Relative Imbibitional Capacities of the Glutens before drying.*—It is well known that when animal proteins are treated with different concentrations of alkali imbibitional effects similar to those in acid are obtained. It was thought desirable, therefore, to see if the glutens from the strong and weak flours showed the marked imbibitional differences noted in acids when the glutens were treated with alkalies of different concentrations.

A series of sodium hydroxide solutions was prepared of the same normalities as those employed in the study of the acids. The experiment was first tried with gluten from B-780 during an imbibition period of 50 minutes. The discs which were placed in N/2 solution became colored a light yellow; they did not swell but seemed to grow smaller, the rough edges becoming rounded off. It was impossible to remove the discs at the end of the imbibitional period, all coherence having been destroyed. The discs which were placed in the concentrations ranging from N/5 to N/25 did not have such a yellow color and the swelling was at first perceptible, but the discs were not coherent enough to be removed for weighing. In the case of the more dilute alkaline solutions the discs were coherent enough to be removed for weighing. Increases in weight were noted for those discs in N/200 and N/500 alkali while decreases were recorded for N/100 and N/50 solutions. The time in alkali was the same as was used for the acids, i. e., 50 minutes. In all solutions but N/500 there was a perceptible cloudiness and a ring of white solid material (probably starch) settled around the discs. This ring was not present in the N/500 solution. In order to ascertain whether or not this was a case of complete

dispersion or solution of the protein, or whether the alkali was merely causing loss of coherence, the solutions of alkali used in the imbibitional experiments were neutralized. A curdy precipitate appeared in each solution excepting N/500, the quantity being roughly proportional to the concentration of the alkali. This showed conclusively that the gluten was in reality being dispersed and by the amount of precipitate one would conclude that the process was going on rather rapidly.

It would seem that the action of alkalies on gluten is somewhat different from the action of the acids. In the case of acids the first step is the swelling of the gluten with no apparent dispersion. This is easily shown by bringing the solution after the removal of the gluten discs at the end of the 50 minute period to the isoelectric point of the gluten. In no instance was there more than a slight turbidity produced. In the case of acid there is marked imbibition as shown by appearance and by the increase in weight. In the case of alkali however the two steps, imbibition and dispersion, follow one another almost immediately so that in some concentrations they compensate each other. In the more dilute solutions the imbibitional factor is the more prominent, in the more concentrated solutions the dispersion factor is the most apparent.

As the imbibition in the case of alkali appeared to be greater in the more dilute solutions, concentrations of sodium hydroxide were tried ranging from N/1000 to N/10,000. No appreciable imbibition was noted however.

In an attempt to make the imbibition factor more prominent the time for the swelling determination in the presence of alkalies was shortened from 50 minutes to 25 minutes. A comparison of the results obtained in these two time intervals is shown in Table XI.

It was concluded from Table XI and the swelling size as shown by appearance that 25 minutes would indicate the imbibition factor more accurately.

The imbibition of the different glutens was studied in solutions of the following alkalies: potassium, sodium, barium,

TABLE XI

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) by Gluten B-780 (before drying) in Different Concentrations of Sodium Hydroxide. Comparison of Imbibitional Periods of 50 and 25 Minutes. (Average of 10 Determinations)

Concentration of alkali	50 minutes	25 minutes
N/100	—0.025	0.088
N/200	0.099	0.242
N/500	0.090	0.033
N/1000	0.010	0.007

calcium and ammonium hydroxides. The data are shown in Table XII and graphically in Fig. 5.

TABLE XII

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Various Glutens (before drying) in Different Concentrations of the Various Alkalies. Imbibition period 25 Minutes. (Average of 5 Determinations)

Concentration of alkali	B-780	B-781	B-782	B-783
Potassium Hydroxide				
N/100	0.138	0.208	—0.084	—0.109
N/200	0.328	0.072	0.008	—0.011
N/500	0.042	0.000	0.018	0.017
N/1000	0.001	—0.023	—0.012	—0.029
Sodium Hydroxide				
N/100	0.088	0.237	—0.039	—0.092
N/200	0.242	0.061	0.059	0.025
N/500	0.033	—0.001	—0.002	0.000
N/1000	0.007	—0.005	—0.014	—0.019
Barium Hydroxide				
N/100	0.065	0.142	0.001	—0.055
N/200	0.114	0.021	0.045	0.023
N/500	0.023	—0.021	—0.022	—0.018
N/1000	0.016	—0.011	—0.022	—0.009
Calcium Hydroxide				
N/100	0.125	0.070	0.025	—0.057
N/200	0.108	0.046	0.041	0.018
N/500	—0.003	0.008	—0.011	—0.013
N/1000	—0.015	0.002	—0.021	—0.016

TABLE XII (continued)
Ammonium Hydroxide

N/100	0.158	0.070	0.067	0.053
N/200	0.080	0.021	0.012	0.013
N/500	0.016	—0.002	—0.015	—0.020
N/1000	—0.011	—0.014	—0.020	—0.012

Fig. 5

The imbibition curves for the various glutes in different concentrations of potassium, sodium, barium, calcium and ammonium hydroxides (before drying, imbibition period 25 minutes).

In all experiments the strong flour gluten, B-780, showed a distinctly greater imbibition in the alkalis than did the gluten from the weak flours, B-782 and B-783. This is par-

ticularly noticeable in the potassium hydroxide series. The differences are more apparent in the N/200 concentrations. In the higher concentrations the dispersion factor is more prominent as shown by the fall in the curve. The clear flour, B-781, does not behave like the others in this respect. The discs from gluten B-780 that were placed in the N/100 alkalis dispersed more than those placed in the lower concentrations. The discs placed in N/200 dispersed somewhat, those in the other two concentrations hardly at all. B-782 and B-783 behaved in all respects like gluten B-780 with the exception that the dispersion was more marked in the case of B-782 and B-783. The clear B-781 did not disperse as readily as did the other three glutens. This would be indicated by the above tables and is shown very clearly in Fig. 5 where the curve for B-781 rises above the others in the N/100 concentration.

(b) *Imbibition of the Dried Glutens in Alkali.*—In the first attempt to prepare moist gluten from the dried material the dried gluten was treated in the same way that the original flour was treated, that is, enough water was added to the dried material to form a stiff dough. This dough was allowed to stand under distilled water for one hour and was then washed in a stream of distilled water. As soon as the washing was begun, however, the gluten began to disperse. This was tried repeatedly with the dried glutens from the different flours. It was found, however, that if a very small amount of sodium chloride was added to the wash water the gluten immediately came together in a coherent mass which showed greater elasticity than the gluten before drying. If the coherent mass obtained by washing in sodium chloride solution was washed in distilled water it began to disperse but was easily brought to a coherent mass by again washing in the salt solution. This process was repeated several times with the same sample and probably could be repeated indefinitely. The method finally adopted was to add enough water to make a dough and let this dough stand under distilled water for one hour. The material was then pressed into sheets without working.

It was found that discs cut from gluten prepared in this manner varied in their behavior to alkali according to the length of time the cut discs stood in water before placing them in the alkali solutions. The following conditions were tested:

A. Discs stood in water overnight before placing in the alkali, B-780.

B. Discs stood in water one hour before placing in the alkali, B-780.

C. Discs stood in water 10-35 minutes before placing in the alkali, B-780.

D. Discs were not placed in water at all, B-780.

E. Added sodium chloride to wash water after trying to wash in distilled water, B-780.

F. Added sodium chloride to wash water after trying to wash in distilled water; the cut discs were allowed to stand in distilled water three hours before placing in the alkali, B-780.

G. Washed with sodium chloride solution; the cut discs were allowed to stand in distilled water overnight. Considerable swelling took place in these discs on standing in water overnight, B-783.

H. Discs stood in water one hour before placing in alkali, B-783.

The gain in weight per gram of moist gluten is given in Table XIII.

TABLE XIII

The Quantity of Water imbibed (Grams per Gram Moist Gluten) for the Glutens B-780 and B-783 (after drying) in Different Concentrations of Potassium Hydroxide, showing the Effect of Different Treatments of Discs of Moist Gluten prepared from Dried Gluten.

Imbibitional Period 25 Minutes. (Average of 5 Determinations)

Concentration of alkali	B-780						B-783	
	A	B	C	D	E	F	G	H
N/100	—	0.052	0.158	0.120	0.193	0.207	0.188	0.163
N/200	-0.111	-0.056	0.007	0.033	0.119	0.112	0.072	0.088
N/500	—	-0.087	-0.045	0.026	0.007	0.036	-0.001	-0.010
N/1000	—	-0.054	-0.019	0.051	-0.019	0.025	0.008	-0.021
Water	—	0.103	0.160	0.124	0.077	0.137	-0.003	0.025

The effect of the different treatments on imbibitional rate is shown very clearly in the N/200 concentration where variations from -0.111 to $+0.119$ are recorded, depending on previous treatment.

The method finally adopted for the study of imbibition of the dried glutens was the same as that employed in the work with dried glutens and acids, i. e., not to place the discs in water at all before placing them in the solutions to be tested. The losses in all cases with alkalis are not attributed to the dehydration of the gluten but rather to its dispersion. After the discs had been removed for weighing the alkali was neutralized and a precipitate was formed which appeared to be approximately proportional to the concentration of the alkali and therefore more or less proportional to the loss in weight of the discs. Although a rather extensive series of experiments was carried out it was found that no method could be devised whereby imbibitional values could be obtained with the dried glutens which were free from large errors due to dispersion. Consequently it is not thought worth while to record the tabular data. The experiments, however, emphasize rather strongly the marked changes in colloidal condition which occur in drying the glutens and confirm the conclusions arrived at in the earlier sections where imbibition of dried and undried glutens in acid solutions is considered.

3. The Effect of Calcium and Potassium Hydroxides containing M/200 Sodium Sulfate on Imbibition

It is well known that neutral salts markedly decrease imbibition of proteins in acid or alkaline media. Gortner and Doherty (I) have already presented data showing that wheat gluten is no exception to this general rule, at least in so far as acids are concerned. In order to ascertain whether or not the same relative effect held true with imbibition in alkali in the presence of salts, experiments were carried out with glutens B-780 and B-783 in the presence of M-200 sodium sulfate in potassium and calcium hydroxides. The results obtained are found in Tables XIV and XV and are expressed graphically in Fig. 6.

Fig. 6

The imbibition curves for the glutens B-780 and B-783 in potassium and calcium hydroxides alone and in potassium and calcium hydroxides plus M/200 sodium sulfate (before drying, imbibition period 25 minutes).

TABLE XIV

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Glutens B-780 and B-783 (before drying) in Various Concentrations of Potassium Hydroxide, and Potassium Hydroxide plus M/200 Sodium Sulfate. Imbibition Period 25 Minutes. (Average of 5 Determinations)

Concentration of alkali	B-780		B-783	
	KOH	KOH + Na ₂ SO ₄	KOH	KOH + Na ₂ SO ₄
N/100	0.138	0.059	−0.109	−0.170
N/200	0.328	0.196	−0.011	−0.043
N/500	0.042	−0.013	0.017	−0.005
N/1000	0.001	−0.020	−0.029	−0.039
Water	—	−0.011	—	−0.031

The results are in good accord with the depressing effect of salts upon imbibition in acids and it was not thought worth while to conduct any further experiments to show the effect of salts inasmuch as their effect has been shown on so many proteins by many different workers. The present series of experi-

ments, however, points to the fact that the difference between a strong and a weak gluten is not merely a question of the presence of more inorganic salts in the weaker gluten.

TABLE XV

The Quantity of Water imbibed (Grams Water per Gram Moist Gluten) for the Glutens B-780 and B-783 (before drying) in Various Concentrations of Calcium Hydroxide, and Calcium Hydroxide plus M/200 Sodium Sulfate. Imbibition Period 25 Minutes. (Average of 5 Determinations)

Concentration of alkali	B-780		B-783	
	Ca(OH) ₂	Ca(OH) ₂ + Na ₂ SO ₄	Ca(OH) ₂	Ca(OH) ₂ + Na ₂ SO ₄
N/100	0.125	0.055	-0.057	-0.140
N/200	0.108	0.131	0.018	-0.033
N/500	-0.003	0.002	-0.013	0.017
N/1000	-0.015	-0.011	-0.016	-0.022
Water	—	-0.011	—	-0.031

4. Relation of Hydrogen Ion Concentration of Various Acids to Imbibition as Measured by Increase in Weight of Discs

Thus far investigators of imbibition, as shown by the increase in weight of discs placed in different concentrations of various acids, have plotted their results against normality (titratable acidity) of the acid solutions. By the use of this method curves are obtained of radically different shapes with the different acids used of which hydrochloric represents the one extreme and acetic acid perhaps the other, as shown in Figures 1 to 5 in the article of Gortner and Doherty¹ and in Figures 1 and 2 of this paper.

Loeb^{20,21,22,23} has recently presented evidence tending to show that the highest osmotic pressure of gelatin and albumin, the highest viscosity of gelatin, the greatest swelling

²⁰ Loeb, J.: Ion series and the physical properties of proteins. I. Jour. Gen. Physiol., 3, 85-106, Fig. 14 (1920).

²¹ Loeb, J.: The proteins and colloid chemistry. Science, 52, 449-456, Fig. 2 (1920).

²² Loeb, J.: Ion series and the physical properties of proteins. II. Jour. Gen. Physiol., 3, 247-269, Fig. 9 (1920).

²³ Loeb, J.: Chemical and physical behavior of casein solutions. Ibid., 3, 547-555, Fig. 6 (1921).

of gelatin, as measured by increase in volume, and the highest viscosity and osmotic pressure of casein solutions is reached at the same hydrogen ion concentrations with the various acids, and furthermore with a given protein the same high point is reached with the various acids, with the exception of sulfuric acid.

The questions as to the relationships existing between imbibition and hydrogen ion concentration were recognized at the time the experimental work by Gortner and Doherty was in progress, but at that time no apparatus for the direct determination of hydrogen ion concentration was available and it was not thought advisable to draw conclusions from hydrogen ion data which had been calculated from the existing tables of physical constants.

We have, therefore, determined by the potentiometric method the hydrogen ion concentration of the various solutions of lactic, acetic, orthophosphoric, hydrochloric and oxalic acids in the dilutions used by Gortner and Doherty. The various solutions were prepared by diluting N/1 solutions of the various acids as determined by titration with NaOH (using phenolphthalein as indicator) with the required amount of distilled water. A Leeds and Northrup potentiometer was used for the measurement of the voltage. A Type R high sensitivity galvanometer was used as a current detector, and the hydrogen electrodes were of the Bailey¹⁸ type. All determinations were made in a constant temperature room kept at 25°. A normal KCl calomel electrode and a flowing junction of sat. KCl were used. The millivolt readings were changed to pH by means of the tables of Schmidt and Hoagland.²⁴

The imbibitional data given by Gortner and Doherty for the "P" (a "strong" patent flour), and the "W₂" (a "weak" flour) were used. The results are given in Table XVI and Fig. 7.

²⁴ Schmidt, C. L. A., and Hoagland, D. R.: Table of pH, H⁺ and OH⁻ values corresponding to electromotive forces determined in hydrogen electrode measurements with a bibliography. University of California Publications in Physiology, 5, No. 4, p. 23-69, March 29 (1919).

Fig. 7

The imbibition curves for a "strong flour," gluten "P" and a "weak flour," gluten "W," in various concentrations of lactic, acetic, ortho phosphoric, hydrochloric, and oxalic acids, plotted with the actual hydrogen-ion concentrations of the solutions in which the discs were immersed as abscissa (before drying, imbibition period 50 minutes).

If we compare the imbibition curves given with the two different flours by a single acid, we find that in each instance the curve for the flour "P" rises higher than the curve for the flour "W." The shape of the curves is very similar, each rising rather rapidly as the acid concentration increases, reaching a flat crest, and then falling. The pH of maximum imbibition as indicated on the abscissa does not appear to be the same for the various acids, the maximum points extending over a range of pH from 3.25 to 2.25. The reason for this difference is probably due to the fact that the

TABLE XVI

Relative Imbibition of Glutens "P" and "W₂," from the Data of Gortner and Doherty, at Various Hydrogen Ion Concentrations of Lactic, Acetic, Ortho-Phosphoric, Hydrochloric and Oxalic Acids

Normality of acid solution	pH of acid solution at 25° C	Grams of water imbibed per gram of moist gluten	
		"P"	"W ₂ "
Lactic Acid			
N/2	1.89	1.01	0.87
N/5	2.11	1.16	0.91
N/10	2.27	1.24	0.83
N/25	2.49	1.22	0.86
N/50	2.67	1.10	0.71
N/100	2.82	0.93	0.46
N/200	3.00	0.82	0.38
N/500	3.23	0.63	0.23
Acetic Acid			
N/2	2.50	1.00	0.61
N/5	2.69	1.05	0.62
N/10	2.85	1.03	0.62
N/25	3.08	1.02	0.54
N/50	3.24	0.93	0.54
N/100	3.39	0.76	0.42
N/200	3.54	0.65	0.33
N/500	3.76	0.48	0.22
Ortho-Phosphoric Acid			
N/2	1.33	0.70	0.79
N/5	1.59	0.93	0.85
N/10	1.79	1.11	0.86
N/25	2.05	1.11	0.79
N/50	2.26	1.04	0.73
N/100	2.49	0.92	0.65
N/200	2.73	0.71	0.47
N/500	3.08	0.53	0.18
Hydrochloric Acid			
N/2	0.46	-0.11	-0.02
N/5	0.81	-0.02	0.05
N/10	1.09	0.17	0.21
N/25	1.46	0.51	0.43
N/50	1.77	0.74	0.51
N/100	2.06	0.83	0.55
N/200	2.35	0.77	0.47
N/500	2.73	0.57	0.34

TABLE XVI (continued)

Normality of acid solution	pH of acid solution at 25° C	Grams of water imbibed per gram of moist gluten	
		"P"	"W ₂ "
Oxalic Acid			
N/2	1.03	0.10	0.13
N/5	1.29	0.32	0.34
N/10	1.52	0.51	0.54
N/25	1.82	0.52	0.53
N/50	2.10	0.65	0.51
N/100	2.36	0.50	0.44
N/200	2.62	0.42	0.38
N/500	3.00	0.33	0.27

hydrogen ion concentration was measured on the pure solutions and does not necessarily indicate accurately the hydrogen ion concentration in that layer of liquid in actual contact with the disc during the whole of the imbibitional period. In the case of the strong acids while giving a relatively high concentration of hydrogen ions at the point of greatest imbibition the titratable acidity is relatively low. For this reason the layer of solution in immediate contact with the disc is soon depleted of hydrogen ions and the actual hydrogen ion concentration of the solution in contact with the disc is therefore much lower than is indicated by a determination in the upper parts of the solution. In order to increase the hydrogen ions in the immediate vicinity of the disc it is necessary that diffusion should act over a relatively great distance. In the case of the weaker acids the high point of the imbibition curve occurs at a normality of acid, as measured by titration, that is, much greater than is the case with the stronger acids. The weak acids by their buffer action tend to replenish the solution with hydrogen ions as fast as depleted by the disc. For this reason the maximum point of imbibition for the weak acids seems in general to occur at a lower hydrogen ion concentration than is the case with the strong acids. We think for this reason that these results tend to show that the point of maximum imbibition of gluten as measured by the increase in weight of discs occurs at practically the same hydrogen

ion concentration for the different acids. This conclusion has been clearly verified by an entirely different method where the errors mentioned above were eliminated. Further data on this part will be included in a later paper.

Discussion

The results on the rate of imbibition of the different glutens in the presence of hydrochloric and lactic acids confirm the findings of Gortner and Doherty. The differences for hydrochloric acids compare very favorably with the results they obtained with this acid. The present values show a somewhat greater variation between the glutens of the strong and weak flours than the differences found by Gortner and Doherty. In the case of lactic acid, the differences between the glutens are much more marked than any found by Gortner and Doherty, the gluten B-780 having almost three times the rate of imbibition of the weakest gluten, B-783, indicating that the "strong" flour is stronger and the "weak" flour weaker than the samples with which Gortner and Doherty worked.

The imbibitional rates of the various glutens in alkalies likewise show marked differences. The behavior of the glutens to alkalies is somewhat different from the behavior in the presence of acids, dispersion beginning almost coincident with hydration in the case of the alkalies. If we compare the results given with potassium and sodium hydroxide in the N/200 concentrations, we find differences greater than any found in the case of the acids. When we compare the reaction of the glutens to the various alkalies, we find that they follow the lyotropic series, potassium > sodium > barium and calcium > ammonium.

The imbibitional rate of the strong gluten, B-780, is markedly reduced by the addition of M/200 sodium sulfate to the potassium hydroxide solution as compared with potassium hydroxide alone. B-783 is also affected but to a less extent, probably due to the low imbibition in the alkali without sodium sulfate. In the case of calcium hydroxide plus M/200 sodium sulfate, the effect with both glutens is less marked.

The effect of the sodium sulfate on the swelling of gluten in potassium and calcium hydroxides is in agreement with the findings of others for the effect of salts on the swelling of animal proteins in alkalies.

In order to more directly compare the effect of acids and alkalies on the imbibitional rate, imbibitional experiments were carried out for 25 minute intervals in solutions of lactic and hydrochloric acids. These results indicate that under the same conditions the effect of the acids is somewhat greater than is the effect of alkalies. Here also the difference between hydrochloric and lactic acids is not so marked as it is in the higher concentrations and the longer intervals of time.

The studies with the dried glutens indicate that a marked change of the colloidal state has taken place in the process of drying. This was also shown by the appearance and texture of the wet gluten prepared from the dried material. The results obtained for the rate of imbibition of the dried material with lactic acid show very clearly that the colloidal structure has been profoundly altered by the drying process, the most pronounced change being in the case of the strong flour gluten B-780. It is of interest to note that drying causes the various glutens to become more alike in so far as all colloidal properties are concerned. The strong gluten in particular shows a lowered imbibitional capacity. This is what might be expected if the imbibitional capacity is due to marked colloidal properties, for we know that alternate freezing and thawing or subjection to alternate moist and dry conditions tends to break up the colloid complexes of a soil, and approximately the same factors are operating in the present instance.

The experiments show that results obtained for the dried material may not be compared to those obtained on the original moist gluten. The object of this investigation was not to investigate differences between glutens before and after drying but to study the glutens from the strong and weak flours, which explains why the drying experiments were not carried farther.

The determination of the hydrogen ion concentration of the solutions of acid used for the imbibitional experiments of Gortner and Doherty tends to show that the optimum hydrogen ion concentration for the swelling of discs occurs at a pH of 3.25 to 2.25. Because the hydrogen ion concentration of the solution is not necessarily the hydrogen concentration of the swelling layer of the disc, and because of the difference in behavior of strong and weak acids the true optimum probably is at a slightly lower hydrogen ion concentration. This indicates that the optimum hydrogen ion concentration of the solution of different acids in actual contact with the swelling discs of gluten lies at practically the same value for both strong and weak glutens and argues for little or no difference in the isoelectric points of the two glutens.

Summary

Certain physico-chemical studies were made on the glutens from four flours, one a high grade patent made from northern spring wheat (B-780), one a clear made from northern spring wheat (B-781), and two flours milled from soft western wheats (B-782 and B-783). The baking tests show that these flours are distinctly different. B-780 is what is known as a strong flour and B-782 and B-783 as weak flours.

The data presented seem to warrant the following conclusions:

(1) The rate of imbibition determined in hydrochloric and lactic acids is in agreement with the findings recorded in the first paper of this series, i. e., the "strong" flour gluten has a much higher rate of imbibition than has a "weak" flour gluten.

(2) There is a marked difference in the rate of imbibition of the different glutens in potassium and sodium hydroxides; the difference while not so apparent is noticeable also in the case of barium, calcium, and ammonium hydroxides. Here again in the case of alkalies the "strong" flour gluten has a higher rate of imbibition than has the "weak" flour gluten.

(3) The reaction of glutens to alkalies appears to be somewhat different from the reaction of the glutens to acids,

dispersion taking place much more rapidly and at lower concentrations in the case of alkalis. Indeed dispersion and imbibition are here almost coincident.

(4) The addition of sodium sulfate to the alkalis of potassium and calcium markedly lowered the imbibitional rate. It does not appear, however, from the shape of the curves that a low imbibitional rate in a weak flour is dependent on or determined by a higher salt content.

(5) Drying the glutens washed from the different flours in a vacuum oven at 45–50° C markedly altered the physico-chemical properties of the glutens, the properties of the different glutens studied becoming more nearly alike. This observation is in complete accord with the theory that the strong gluten is strong because of more pronounced colloidal properties inasmuch as it is well known that alternate wetting and drying a colloidal gel breaks down the gel structure.

(6) All of the data in the present paper confirm the postulation of Gortner and Doherty that a weak flour which owes its inferior strength to the *quality* of its gluten is weak because of the fact that its gluten possesses markedly inferior colloidal properties and is not so perfect a colloidal gel as is the gluten of a strong flour.

(7) Evidence is presented tending to show that the optimum hydrogen ion concentration for the imbibition of discs of gluten is the same for the various acids.