reagent had been entirely oxidized as there was no deepening of color when $K_3Fe(CN)_6$ was added.

Summary.

From these experiments we conclude that the phenolphthalin is carried up the xylem and accumulates in the phloem when allowed to stand. It seems clear to us that there must be oxidation going on in the living cell and that there is active oxygen in the protoplasm, since the phenolphthalin was completely oxidized to phenolphthalein in the cells of the plants used.

LEXINGTON, KY.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE KANSAS STATE AGRICULTURAL EXPERIMENT STATION.]

NITROGEN IN AMINO FORM AS DETERMINED BY FORMOL TITRATION, IN RELATION TO SOME OTHER FACTORS MEASURING QUALITY IN WHEAT FLOUR.

BY C. O. SWANSON AND E. L. TAGUE.

Received December 18, 1916.

That nitrogen in amino form[1] has an important relation to the factors which are used to measure the quality of wheat flour, has been shown in several experiments made in this laboratory. Break flour made from sound wheat has a larger percentage of nitrogen in amino form than patent flour made from the same wheat.[2] The break flour has a larger percentage of the material of the wheat kernel next to the bran and the germ than have the patent flours. This is particularly true of the flour streams from the last breaks. The break flour is sound and strong, but is classed as inferior in grade to the patent on account of dark color, high ash, and high acidity.

The percentage of nitrogen in amino form is greater in flour made from germinated wheat than in flour made from the same lot of wheat not germinated. In previous work[2] a slight increase in the amount of nitrogen in amino form gave a greater loaf volume, but a large increase in this form of nitrogen gave a very inferior quality of bread. The addition of amino compounds to the flour in the form of pure chemicals was distinctly detrimental to the resulting bread.[3] In the same experiment it was found that the addition of some chemicals containing the ammonium group was beneficial. It was also found that the extract from bran containing sprouts from germinated wheat was harmful, while the extract from the

[1] Nitrogen in amino form in flour, referred to in citations from publications from this laboratory prior to 1916, mean: nitrogen in nitrogenous compounds soluble in a 1% solution of sodium chloride, and not precipitated by phosphotungstic acid.

[2] Kans. Expt. Station, *Bull.* 177, p. 145.

[3] *Ibid.*, 190.

bran of sound wheat was beneficial. The harmful effects were of the same nature as those produced by amino compounds used as pure chemicals.

The percentage of nitrogen in amino form in mill stream flours varies with the percentage of acidity, ash, and phosphorus.[1] The process of flour milling consists of a series of crushings and siftings. The flour obtained by sifting after each crush is known as a flour stream. The mechanical system of separation is founded on the fact that the bran coat and the germ are tougher than the endosperm. The endosperm contains a smaller percentage of protein and ash than the bran coat. Because the bran coat contains over ten times the percentage of ash found in the endosperm, the amount of ash in the flour depends largely on the completeness of the separation.

The determinations of ash and acidity are two of the chemical tests most widely used in measuring quality in flour. The percentage of ash in flour depends more on the method of milling than on small variations in the ash content of wheat. The same statement is true of acidity, if the flour is made from sound wheat. A flour with a high percentage of ash and phosphorus will have a high percentage of acidity.[2] A small amount of the bran coat in the flour will increase the amount of these substances, but the presence of this small amount of bran is not by itself harmful, as is known by the user of Graham flour. The percentage of ash, acidity, and phosphorus in the wheat is not an indication as to the amount of these substances in the flour made from the wheat.[3]

In previous work[4] it was shown that the amount of amino compounds in flour from germinated wheat increased proportionately to the length of time the wheat had germinated. The per cent. of water-soluble phosphorus did not show any uniform increase proportionate to the length of time germinated, but did vary as the amount of ash and total phosphorus. Water-soluble phosphorus referred to here is the phosphorus in compounds present in the filtered extract.

In the analysis of a series of mill stream flours[5] all made from the same sound wheat, it was found that the percentage of amino compounds in the original wheat was 0.530; in the patent flour, 0.162; in the clear, 0.270; and in the low grade, 0.396. The patent flour consists almost wholly of material from the inside of the wheat kernel. The clear flour contains more of the endosperm next to the bran. The low grade contains also some finely pulverized bran particles. Since the amino compounds are found in the endosperm of sound wheat, they are normally present in

[1] Kans. Expt. Station, *Bull.* 202; *J. Ind. Eng. Chem.*, 4, 274 (1912).

[2] Kans. Expt. Station, *Bull.* 202.

[3] *Ibid.*, 66–76.

[4] Kans. Expt. Station, *Tech. Bull.* 1, 51–54.

[5] *J. Ind. Eng. Chem.*, 4, 274 (1912).

all flour, the better grades containing the smaller amounts. It is only when present in large amounts that they indicate inferiority.

A comparatively large percentage of ash and acidity does not by itself denote unsoundness. Since the bran and shorts contain ten times as much ash, and about eleven times as much acidity as the flour,[1] the presence of a very small amount of bran coat will greatly increase the ash and acidity value.

Experimental.

In using the formol titration according to Sörensen[2] in measuring the rate of protein hydrolysis due to the enzymes present in flour, it was noticed that the number of cc. of 0.05 N $Ba(OH)_2$ used in titrating for acidity (in order to get a neutral starting point) showed more or less correlation to the number of cc. used in neutralizing after the addition of the formaldehyde. This relationship varied somewhat with the grade of flour used. In order to test this more thoroughly, a number of samples of flours were collected from different mills, and also one set of mill stream flours from a medium-sized mill. These flours were analyzed for ash and total nitrogen according to the usual methods, and they were also analyzed for amino nitrogen according to the following method: Water and flour were mixed in a proportion of 10 : 1 and kept in a water bath for two hours, at 40°. The mixture was shaken once every fifteen minutes, and then after settling it was filtered. Portions were then titrated for the first and second stage,[3] measuring acidity and amino nitrogen, respectively.

Results on Mill Stream Flours.

The results on mill stream flours are given in Table I. According to a statement from the mill, the percentage of patent was 65, and the clear 35. The patent flour was made up of the first, second, third, fourth, fifth, and sixth middlings, a special stream, and the first sizings, while the clear was the remainder of the streams. The clear in this case would contain the streams which sometimes go into the low grade, such as the ninth middlings and the tailings. The straight was made up of a mixture of the patent and the clear. In Table I the flour numbers followed with a P denote the streams which go into the patent, while C indicates those that go into the clear. The streams which go into the patent are fewer, but contain more flour than those which make up the clear.

All the results are given on the basis of 10 g. of flour: nitrogen and ash in milligrams, acidity and amino nitrogen in cc. of 0.05 N $Ba(OH)_2$. The results are in ascending series, using the ash figures as a basis. This arrangement is used in all the tables.

[1] Kans. Expt. Station, *Bull.* **202**, 69–72.

[2] This Journal, **38**, 1098–1109 (1916).

[3] *Loc. cit.*

The average acidity figure for the streams making up the patent flour is 2.20. The average of the streams going into the clear is 5.16. There is little variation in the streams which go into the patent, hence the figures obtained for the patent flour itself is practically identical with the average of the streams of which it is made. The mill stream flours which make up the clear vary a great deal in acidity value. If the same amount of flours were obtained from each stream then the average of the values obtained from these different streams should be the same as that obtained from the clear flour, but some of these streams give a very small amount of flour, and those giving the smaller amounts usually have the greater acidity value, hence the average of the individual streams is greater than that of the sample of clear flour.

The average of the figures for the formol titration on the streams which make up the patent flours is 3.04. That of the sample of patent flour is 2.95, an insignificant difference, but if the largest figure, 3.85, which was obtained from the sixth middling, one of the smaller streams, be omitted from the average, then the average is 2.97. The above discussion shows that the formol titration for nitrogen in amino form has a value comparable with the titration for acidity in measuring the quality of the streams which should go into the patent.

The average of the figures for the formol titration on the streams which make up the clear grade is 4.58, a little larger than what was obtained from the clear flour itself. This is due to the fact that some of the streams which make up a small percentage of the total volume have a larger amount of amino nitrogen than those which make up the larger portion of the flour.

It is notable that the figures for the formol titration on the streams which make up the patent flour are larger in every case than the acidity figures but for the clear flour, with the exception of second and third break, grader, tailings and sizings, the figures for the acidity value is higher than the formol titration value. The average figures for acidity and formol titration on these streams are high as compared with the average of the streams which go into the patent.

The figures given in Table I are plotted in related curves. In plotting these curves the centigrams of ash and total nitrogen are used as units. This makes figures more comparable with the cc. expressing the acidity and formol titration value. The numbers on the abscissa are the numbers of the flour samples. (See Plate I.) The ash and acidity curves follow each other closely. The curve for formol titration follows the acidity curve on the upper side uniformly for all the streams which go into the patent, and with the exception of five streams of the clear, the formol curve is on the lower side. At the present time the ash content and the color value are two of the most important factors which decide whether

PLATE I
MILL STREAMS

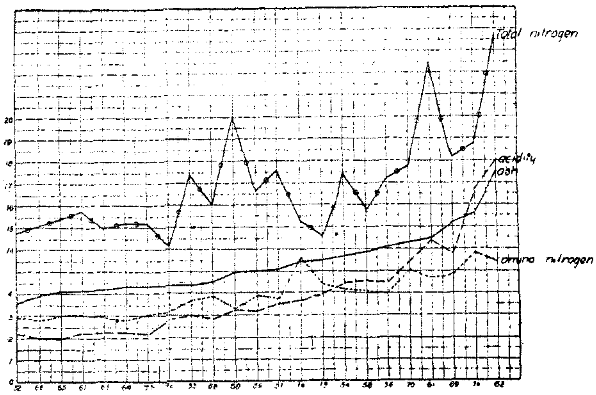


PLATE II
PATENT FLOUR

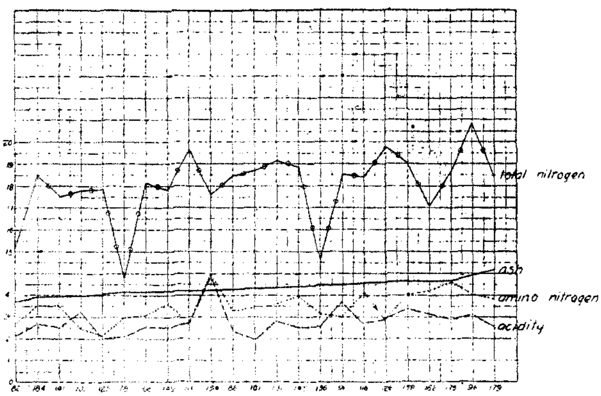


PLATE III
STRAIGHT FLOURS

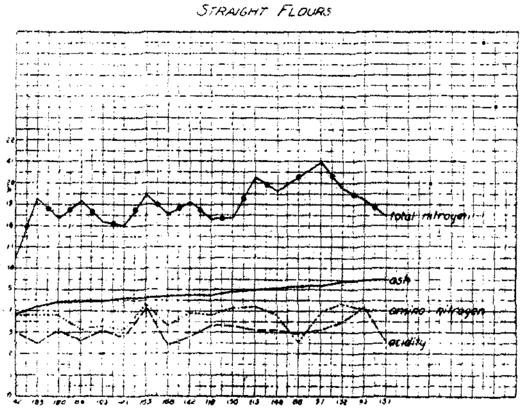


PLATE IV
CLEAR FLOURS

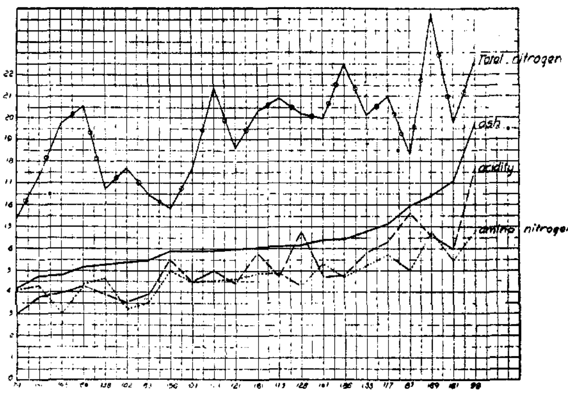
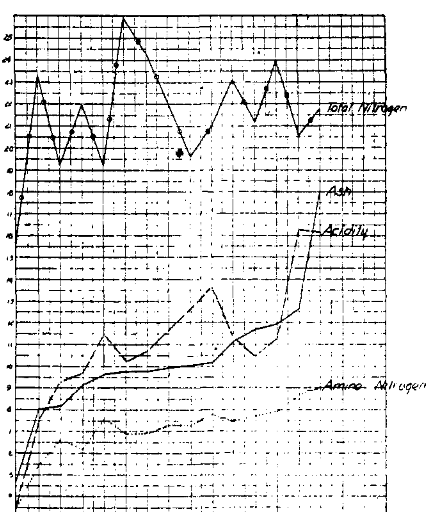


PLATE V
LOW GRADE



a stream shall go into the patent or into the clear. These are to a large extent matters of taste and education. A high percentage of ash *per se* does not mean inferiority. It simply means that the flour streams containing bran portions of the wheat kernel are incompletely separated from the others. Some streams which contain large amounts of endosperm next to the bran, such as the fifth break, have a yellow or gray color. The dietetic value of these flour streams is not inferior to those streams where the color is less pronounced. The conditions of trade have fixed a lower commercial value on flours where this color occurs. The formol titration assigns a higher value to several streams which go into the clear than does the determination of ash, or the determination of acidity.

Results on Commercial Flours from Different Mills in Kansas.

In general, each mill providing samples for this investigation, sent such as they were making at the time, usually patent, straight, and clear. A number of mills were also making a low grade. These flours were analyzed for the same constituents as were the mill stream flours. The results are grouped in Tables II, III, IV and V, and the arrangement of the results follows the same scheme as in Table I. The related curves are found in Plates II, III, IV and V. All the patent flours except three, and all the straight flours except two, have a higher figure for the formol titration than for the acidity. Of the twenty-four clear flours, eleven have a figure for the formol titration higher than the acidity figure. All the low grade flours give a higher figure for the acidity than for the formol titration.

If the magnitude of the figures for ash, acidity, and formol titration in the four classes of flours are compared, it is seen that the classification is more or less arbitrary. From the analysis, it is not possible to say whether a flour is a clear or a straight, except in a general way that figures for ash, acidity, and formol are higher in the straight, but many individual

TABLE I.—MILL STREAMS.

Flour No.	Kind.	Ash, mg. in 10 g.	Acidity cc. 0.5 N $Ba(OH)_2$ for 10 g.	Formol cc. 0.5 N $Ba(OH)_2$ for 10 g.	Total N mg. in 10 g.
52 P	Patent flour	36.4	2.21	2.95	148.00
66 P	4th middlings	39.2	2.00	2.79	150.00
65 P	3rd middlings	41.0	1.99	2.98	153.90
67 P	5th middlings	41.2	2.23	3.07	158.00
63 P	1st middlings	41.6	2.13	2.96	150.10
64 P	2nd middlings	43.0	2.20	2.80	152.00
55 P	Special stream	43.6	2.14	3.04	151.35
72 P	1st sizings	43.6	2.87	3.17	141.40
53	Straight flour	43.8	3.00	3.70	173.00
68 P	6th middlings	45.4	2.83	3.85	160.20
60 C	3rd break	49.6	3.22	3.23	200.00
59 C	2nd break	50.0	3.20	3.90	165.70
57 C	Grader	50.4	3.50	3.75	176.00
74 C	Fine tailings	54.6	3.68	5.67	153.60
73 C	2nd sizings	54.8	3.94	4.36	146.50
54 C	Clear flour	56.6	4.43	4.17	174.75
58 C	1st break	58.2	4.60	4.10	158.00
56 C	Reel No. 2	61.2	4.48	3.96	174.75
70 C	8th middlings	62.6	5.39	5.06	177.85
61 C	4th break	64.9	6.40	4.73	223.80
69 C	7th middlings	72.1	5.87	4.86	181.60
71 C	9th middlings	75.9	7.65	5.85	187.80
62 C	5th break	96.3	10.05	5.48	237.90

TABLE II.—COMMERCIAL PATENT FLOURS.

Flour No.	Ash, mg. in 10 g.	Acidity 0.05 N $Ba(OH)_2$ for 10 g.	Formol 0.05 N $Ba(OH)_2$ for 10 g.	Total N mg. in 10 g.
82	37.20	2.15	2.72	152.10
184	39.20	2.70	3.50	184.80
141	39.50	2.50	3.50	174.85
101	40.00	3.20	2.48	177.70
125	40.20	2.00	2.10	177.70
78	41.20	2.10	2.98	138.10
106	41.40	2.50	3.10	181.25
149	41.50	2.50	3.70	177.90
111	41.60	2.70	2.60	197.10
154	42.00	4.90	5.00	176.30
86	42.80	2.38	3.32	184.40
167	43.10	2.00	3.40	186.00
131	43.60	2.80	3.60	191.10
145	44.00	2.50	4.00	187.80
136	44.60	2.50	3.10	147.10
91	44.70	3.60	3.00	185.20
116	45.40	2.72	4.18	184.35
126	45.80	2.80	2.90	196.85
159	46.30	3.40	4.00	191.00
162	46.45	3.10	4.20	172.00
175	46.70	2.90	4.60	186.40
96	49.30	3.10	4.06	207.90
179	51.30	2.60	3.90	184.60

TABLE III.—COMMERCIAL STRAIGHT FLOURS.

Flour No.	Ash, mg. in 10 g.	Acidity 0.05 N $Ba(OH)_2$ for 10 g.	Formol 0.05 N $Ba(OH)_2$ for 10 g.	Total N mg. in 10 g.
142	38.80	3.00	3.90	165.35
185	41.90	2.50	3.80	192.05
180	43.80	3.10	3.80	184.10
84	44.00	2.63	3.17	190.75
103	44.40	3.10	3.30	181.50
127	45.80	2.80	3.10	180.30
155	45.80	4.10	4.30	194.90
168	46.60	2.40	3.30	185.00
172	47.40	2.80	3.90	190.50
118	47.50	3.30	3.80	182.70
150	48.50	3.30	4.10	183.60
113	49.60	3.10	4.20	203.00
146	50.30	3.10	3.80	196.00
88	51.00	2.92	2.58	158.20
97	51.00	3.15	3.95	209.30
132	53.80	3.50	4.30	198.10
93	54.70	4.16	4.04	192.25
137	54.80	2.60	4.00	184.60

TABLE IV.—COMMERCIAL CLEAR FLOURS.

Flour No.	Ash, mg. in 10 g	Acidity 0.05 N $Ba(OH)_2$ for 10 g.	Formol 0.05 N $Ba(OH)_2$ for 10 g.	Total N mg. in 10 g.
79	42.40	3.00	4.02	154.30
151	47.30	3.80	4.30	173.35
143	47.60	4.00	3.00	197.20
164	51.30	4.30	4.40	205.10
138	52.80	4.00	4.60	166.90
102	53.50	3.54	3.26	176.70
83	54.40	3.90	3.50	163.90
156	58.90	5.50	5.00	158.60
107	58.90	4.50	4.50	177.60
177	58.90	5.00	4.50	213.00
121	60.00	4.40	4.60	186.60
161	60.60	5.80	4.90	203.50
173	61.10	4.70	4.90	209.25
128	61.80	6.80	4.30	202.10
147	64.60	4.70	5.40	200.30
186	64.60	4.80	4.70	225.30
133	68.20	5.80	5.20	201.00
117	71.20	6.30	5.70	210.00
87	80.00	7.61	5.02	183.20
169	84.40	6.60	6.80	247.80
181	90.00	6.00	5.50	198.30
98	118.20	9.68	6.82	226.10

TABLE V.—COMMERCIAL LOW GRADE FLOURS.

Flour No.	Ash, mg. in 10 g.	Acidity 0.05 N $Ba(OH)_2$ for 10 g.	Formol 0.05 N $Ba(OH)_2$ for 10 g.	Total N mg. in 10 g.
80	47.00	3.60	3.48	156.50
187	80.20	7.50	5.50	232.50
139	81.80	9.30	6.70	192.50
104	91.40	9.72	6.18	218.80
152	96.40	11.50	7.60	191.90
134	97.80	10.30	6.90	258.60
174	97.80	10.70	6.90	242.90
157	99.50	11.70	7.30	218.60
129	100.30	12.70	7.30	196.60
182	101.60	13.70	7.80	210.30
99	111.40	11.28	7.52	231.00
123	117.40	10.50	6.40	211.75
109	119.80	11.30	7.90	239.70
89	162.20	16.21	8.71	205.60
94	180.00	16.10	9.00	218.00

straight flours give a better test as far as these figures go, than many patents. A few of the clear flours give as low figures for ash, acidity, and formol as most of the patents and straights, but the outstanding result as far as the clear flours go is the lack of uniformity, and the extreme variation. This last statement holds true also in regard to the low grade.

Discussion and Summary.

Previous work done in this laboratory has shown that a comparatively large amount of nitrogen in amino form, as determined by the method of precipitation with phosphotungstic acid, is an indication of certain undesirable qualities in flour. These qualities in sound flour are of the same kind as those denoted by ash and acidity. Nitrogen in amino form, as measured by formol titration, is valuable together with the determination of ash and acidity in measuring quality in flour. This difference, however, should be noted: Titrable nitrogen is more uniformly distributed in the wheat kernel than are the materials which determine the amount of ash and acidity. Therefore, in clear and low grade flours, as compared with patent and straight flours made from the same sound wheat, the increase in titrable nitrogen is not proportionate to the increase in ash or acidity.

The relations of chemical constituents to the factors which determine quality in flour are not well known. The titrable nitrogen measures a certain degree of protein hydrolysis, or the presence of nitrogenous substances, similar in nature to those produced by protein hydrolysis. These substances are the ones which are indicated by the titrable nitrogen in flour from sound wheat. If wheat has been subjected to unfavorable conditions to such an extent that the proteolytic enzymes have caused splitting of the protein, the amount of titrable nitrogen would be increased. This is a field worthy of investigation. The chemical factors which determine quality in gluten are little known. The data in the present paper are offered as a contribution to the general problem. More extensive work must be done before definite conclusions can be drawn. The data show that the lower grades of flour such as the clear and low grade, made from sound wheat, do not contain nitrogenous substances such as are measured by the formol titration, in as large a proportion as ash and acidity.

[Contribution from the Laboratory of Agricultural Chemistry of the University of Wisconsin, Madison.]

THE PHYTIC ACID OF THE WHEAT KERNEL AND SOME OF ITS SALTS.

By P. W. Boutwell.[1]

Received December 26, 1916.

Part I.—Attention has often been called to the marked lack of uniformity which exists in the analysis of phytic acid and of its salts which have been published from time to time. An explanation of these divergent results ob-

[1] Abstract of a thesis submitted to the Graduate Faculty of the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy.