given below may therefore differ from the true ratios by that amount. In the experiments tabulated below, somewhat greater concentrations were used than in the previous experiments, in order that the iodide present might become a measurable quantity.

TABLE III.

Equilibrium $AgBr + KI \rightleftharpoons AgI + KBr$ at $25°$.

No.	Approx. Concen.	Reacting salts.	Conc. Br.	Conc. I.	$\sqrt{\dfrac{C_i}{C_{Br}}}$
1a	N	AgI + KBr	0.955	$24.4 \times (10)^{-5}$	0.016
1b	N	AgBr + KI	0.945	$13.9 \times (10)^{-5}$	0.012
2a	N/5	AgI + KBr	0.185	$4.83 \times (10)^{-5}$	0.016
2b	N/5	AgBr + KI	0.187	$2.2 \times (10)^{-5}$	0.011

Mean ratio $\dfrac{S_{AgI}}{S_{AgBr}} = 0.014$

From the ratios recorded in Tables I, II and III the relative solubility of the whole series may be calculated. The values thus obtained are given in Table IV. Column 1 designates the salt, column 2 its relative solubility referred to that of silver chloride as unity, and column 3 the absolute solubility in gram-molecules per liter, taking Kohlrausch and Rose's[1] figure for the silver chloride as the standard. Column 4 gives the maximum and minimum values obtained for the solubility of these salts by other methods; the figures are taken from the table complied by Abegg and Cox.[2]

TABLE IV.

Salt.	Relative solubility.	Absolute solubility.	Extreme values.
AgCl	1.00000	$1.6 \times (10)^{-5}$	$1.25—1.64 \times (10)^{-5}$
AgCNS	0.07480	$1.2 \times (10)^{-6}$	$1.08—1.25 \times (10)^{-6}$
AgBr	0.05500	$8.8 \times (10)^{-7}$	$6.6 —8.1 \times (10)^{-7}$
AgI	0.00077	$1.23 \times (10)^{-8}$	$0.97—1.05 \times (10)^{-8}$

The foregoing investigation is one of a series planned in collaboration with Professor M. A. Rosanoff. A change of residence has made it advisable to carry out the separate parts of the work independently. Credit is gladly given to Professor Rosanoff for many of the ideas contained in this paper.

NEW YORK UNIVERSITY,
October, 1907.

ON THE ANALYTICAL ESTIMATION OF GLIADIN.

BY WALTER E. MATHEWSON.

That the common methods for the determination of gliadin are unsatisfactory is generally recognized. The amount of nitrogenous material

[1] Abegg and Cox, Z. physik. Chem., 46, 11 (1903).
[2] Loc. cit.

extracted by dilute alcohol varies with the strength of alcohol used,[1] the relative proportion of alcohol to the flour,[2] and with the relative amounts of certain non-nitrogenous constituents of the latter, as acids[3] and salts. The experiments described here were carried out in the hope that a better procedure might be devised than that ordinarily followed. Five of the flour samples used were patent flours milled in the Experiment Station with the Experimental Roller Reduction Mill by Mr. C. O. Swanson. They had been bolted through No. 10 and No. 12 cloth. The sixth sample (No. 6) was a patent flour from the Manhattan Milling Company.

All alcohol used had been redistilled before use and was perfectly neutral to sensitive litmus paper. The concentrations given are by weight, not by volume. The alcohol, phenol, etc., used in the nitrogen determination were tested by blanks and the phenol was also examined for optical activity. All extractions were made in duplicate, the maximum difference allowed on the nitrogen determinations being 0.03 per cent. The results are expressed in terms of crude gliadin, obtained by multiplying the nitrogen found in the extract by 5.7. No attempt was made to correct for the amides present, since the values obtained in this determination are so largely dependent . upon the protein precipitant used.[4] They could have been present only in very small amount, as the flours were sound and fresh. All percentages given are based upon the air-dry substance. The validity of the views of Osborne and Voorhees regarding the wheat proteins is assumed throughout the discussion.[5]

Below are given the percentages of moisture, crude protein (nitrogen $\times$ 5.7), and crude gliadin of the samples as determined in one of the usual ways; namely, by extracting 4 grams of the charge with 100 cc. cold 70 per cent. alcohol in a tight bottle, filtering, and determining nitrogen in 50 cc. of the filtrate. In the last column are given the results obtained when 16 grams of flour to 100 cc. of alcohol were taken.

No. of Sample	Moisture.	Total crude protein.	Crude gliadin. 4 grams flour, 100 cc. alcohol.	Crude gliadin. 16 grams flour, 100 cc. alcohol.
1	11.20	11.42	6.00	5.08
2	11.70	7.21	3.72	3.19
3	11.23	11.20	5.56	4.90
4	10.90	11.17	5.92	5.34
5	11.52	12.61	6.75	5.93

To guard as far as possible against incomplete extraction the mixtures

[1] Teller, Bull. Ark. Expt. Sta., No. 53, p. 63.

[2] Chamberlain, Bull. Bur. Chem., U. S. Dept. Agr., p. 125, No. 90. Chamberlain, This Journal, 28 (1906), 11, p. 1660.

[3] Snyder, Annual Rept. Minn. Expt. Station, 1904, p. 206.

[4] Schulze, Landw. Vers.-Sta., 26, 213 (1881).

[5] Osborne and Voorhees, Am. Chem. J., 15, 392 (1893).

were allowed to digest about forty-eight hours before filtration, being begun in the afternoon and frequently shaken until evening and during the next day. The fear that any gliadin would be coagulated by prolonged contact with 70 per cent. alcohol seems to the writer to be entirely unfounded, as pure gliadin prepared by Osborne's method, dissolves completely in alcohol of this concentration and the solutions remain clear for weeks, perhaps indefinitely. Nor would one anticipate that the error due to solution of nitrogenous matter not gliadin would be increased much relatively by the standing during the second day.

Sample No. 6 when treated with varying amounts of solvents gave the following results:

Grams flour per 100 cc. alcohol.	Per cent. crude gliadin.
1	4.57
2	4.39
4	4.37
8	4.29
16	4.22

The lower results obtained when the larger quantities of flour are used may be due both to less complete extraction and to the solution of nongliadin nitrogenous substances, which dissolve but slightly and in more or less constant amount.

Gluten appears to be a solid colloid solution containing essentially the two gluten proteins with water. It would seem possible that a certain amount of gliadin might be held in solution in the glutenin, tending to divide itself between the two phases according to the distribution law. To test this a solution of pure gliadin in alcohol of about 60 per cent. was prepared. It gave a rotation of 21.6° Ventzke in a 200 mm. tube in a triple shadow saccharimeter. Twenty-five cc. of this solution were added to 5 grams of gliadin-free, dried flour prepared from sample No. 6 by repeated extraction with dilute alcohol, washing with concentrated alcohol and drying. The mixture was shaken frequently for three or four hours, allowed to stand over night, filtered and the filtrate polarized. A sample of the gliadin solution was also filtered under the same conditions, as a check to determine possible loss by evaporation under these conditions, and another sample of the flour was digested in the same way with 70 per cent. alcohol, to make sure that optically-active, alcohol-soluble substances had been removed. The gliadin solution was found to have suffered no change in concentration that could be detected with the saccharimeter, by contact with the flour. A second experiment was carried out in exactly the same way except that instead of flour, 5 grams of air-dry, pulverized crude glutenin were used, this being prepared by treating thoroughly washed gluten, cut into small pieces in a meat cutter, with successive portions of dilute alcohol for some time. It was then

washed with strong alcohol, allowed to dry at room temperature, ground to a powder that would pass through a 1 mm. mesh sieve, and this extracted in an extraction apparatus with ether and with absolute alcohol. After repeatedly extracting again with dilute alcohol, it was rinsed with strong alcohol and dried at room temperature.

The crude glutenin swelled up in the gliadin solution, but after applying the small correction for increase in concentration on filtration, the rotation was found to have increased from 10.8° V. to 12.1° V. Instead of removing gliadin from the solution the glutenin had evidently taken up water or dilute alcohol, thus increasing the rotation of the liquid. If glutenin had a marked tendency to hold gliadin in solution one would hardly expect this result.

As has been shown conclusively by Chamberlain[1] dilute alcohol dissolves other protein substances from flour beside Osborne's gliadin. Gliadin dissolves most readily in alcohol of about 70 per cent. (by volume), but a weaker alcohol dissolves a considerably larger percentage of nitrogenous matter from flour. Had we some method of rendering this foreign protein matter insoluble before the extraction, without affecting the gliadin, it would remove one of the most important sources of error. It was thought by the writer that possibly this could be effected by heat. A sample of pure gliadin was heated for six hours in an ordinary drying oven surrounded by boiling water. It did not seem to be changed physically and dissolved to a perfectly clear solution on warming somewhat with dilute alcohol, the solution remaining clear on cooling. Nor did gluten made from flour heated in the same way appear to differ much either in properties or amount from that made from similar flour which had not been heated. According to Osborne, leucosin, the albumen of wheat, is coagulated at temperatures of about 55–65°. The globulin is partially coagulated at 100°. Proteoses and amides are present in very small amount.

Below are given the results obtained by extraction with 70 per cent. alcohol after previous heating for five hours in an oven surrounded by boiling water. In the first column are given the results obtained by treating with cold alcohol (100 cc. for 4 grams) in the ordinary way.[2] In the second set of determinations the same proportion of flour and solvent were used, but the bottles were maintained at a temperature of 60–70° for about four hours during the digestion, the hot mixture being frequently shaken. As in the other determinations the flour remained in contact with the alcohol about forty-eight hours, during 12 to 14 of which the mixture was frequently shaken.

[1] Chamberlain, This Journal, 28, 1661 (1906).

[2] The much lower results obtained by Chamberlain are doubtless due in large measure to the difference in time allowed for extraction.

No. of sample.	Crude gliadin, cold extraction.	Crude gliadin, hot extraction.
1	4.97	5.62
2	3.24	3.60
3	4.78	5.46
4	5.30	5.85
5	5.94	6.66

As there seemed some danger that the glass-stoppered digestion bottles might not altogether prevent the loss of alcohol during the heating, they were weighed at the beginning and the close of the digestion. The highest loss was 0.3 gram, most of them not having lost half this amount.

In the opinion of the writer it is impossible to obtain satisfactory results by washing a flour repeatedly on a filter with hot alcohol of the usual concentration. A filter that will retain the fine suspended matter cannot act rapidly and in the washing, it is practically impossible to prevent losses of alcohol from the solvent, as a result of which it is enabled to dissolve more protein.

The percentages of gliadin found by the cold extraction of the dried flour are likely too low, the strongly dried protein going into solution very slowly in the cold solvent. The amounts dissolved in the hot extraction are almost as high as those given by cold alcohol on air-dry flour. If a purer gliadin was dissolved after the heating, the tendency to higher results by the ordinary method must be more or less offset by incomplete extraction.

It was thought that perhaps dilute n-propyl alcohol might prove a suitable solvent for the separation of gliadin. Seventy per cent. propyl alcohol is a mixture of constant boiling-point (about 86°) and hence can be used in an extraction apparatus. Seventy per cent. propyl alcohol dissolves pure gliadin with some difficulty when cold, but readily when hot. The results given below were obtained by using the solvent in a percolating extractor, the 2-gram charge of flour being held in a S. and S. filter paper shell. The liquid in the flask was kept rapidly boiling to bring about as rapid an extraction as possible.

No. of sample	Duration of extraction, hours.	Percentage crude gliadin.
1	10	5.99
2	5	3.66.
3	10	5.51
4	10	6.27
5	10	6.18

The figures indicate no special advantage over the preceding methods.

Anhydrous phenol dissolves gliadin readily and apparently without affecting it chemically.[1] Further, pure phenol has a comparatively limited power as a solvent, and gliadin has a high specific rotation in this

[1] This Journal, **28**, 1483 (1906).

liquid.   Mixtures of flour with phenol are difficult to filter and in making the experiments described below this was accomplished by using a Gooch crucible with asbestos felt.   The filtering tube containing the crucible was connected with a 100 cc. distilling flask which served as the filtering flask.   The pump was turned on and the mixture poured on the felt. A good vacuum was almost at once produced in the little filtering flask. A pinchcock was then placed on the rubber tube leading to the pump and, the apparatus being tight, it was not usually necessary to exhaust it again.   The Gooch crucible was covered with a watch-glass and surrounded by a block-tin coil, through which steam could be passed to keep its contents from congealing.   With this arrangement the filtrations could be made without any perceptible loss of solvent.   Ten-gram charges of the flour were weighed out, dried and extracted with accurately measured volumes of phenol (96 cc. for sample one, 100 cc. for the others), the mixtures being kept at about 40° and frequently shaken for the first three or four hours.   After about twenty-four hours they were filtered.   Nitrogen was determined in an aliquot part by placing it in a Kjeldahl flask, adding about 300–400 cc. of water, 2 cc. of concentrated sulphuric acid and a little granulated zinc.   It was then boiled until only a few cubic centimeters remained, the water vapor carrying off practically all the phenol, but the sulphuric acid preventing any loss of nitrogen as was indicated by the concordance of duplicates.   Sulphuric acid was then added and the determination carried out in the usual way. In calculating the percentage of crude gliadin from the polariscope readings the optical rotation of gliadin was taken $[\alpha]_D^{40°} = 132°$.

CRUDE GLIADIN EXTRACTED BY ANHYDROUS PHENOL.

No. of sample	Crude gliadin calculated from nitrogen determination.	Crude gliadin calculated from polariscope reading.
1	9.86	8.08
2	6.19	4.83
3	9.43	7.93
4	7.12	6.39

The high results and differences between the percentage of crude gliadin as determined by polarization and by the nitrogen determinations show the presence of another protein or proteins largely soluble in phenol. The alternative is that dextro-rotatory substances were also present in the extract.   Portions of the latter from samples 2 and 3 were taken, treated with one-fifteenth their volume concentrated sulphuric acid and the gelatinous precipitates filtered off.   Both filtrates seemed to contain a trace of dextro-rotatory substance, but in so small amount that its presence could not be demonstrated with certainty—certainly less than would correspond to 0.4 per cent. protein in the flour.   Duplicate nitrogen determinations were made on the filtrate from No. 4; it was free from

nitrogen. It is unlikely that any carbohydrate-like substances that might have been dissolved in the phenol would have been precipitated by the sulphuric acid. Gliadin solutions in phenol give no precipitate with phenol solutions of mercuric iodide or of iodine.

The above facts taken in connection with the fact that the gliadin apparently remains unchanged in phenol are of some interest because the view has been advanced that gliadin is formed by the action of dilute alcohol on the wheat, being split off from some other protein substance. The high percentage of protein removed by the phenol evidently includes another protein or other proteins in considerable quantity. An attempt to gain more data regarding this was made by extracting flours with dilute alcohol until the washings were practically free from protein. By this washing any dextro-rotatory substances soluble in dilute alcohol would also be removed. These samples were then dried and extracted with anhydrous phenol, the nitrogen content and the rotation of the extract determined. The amounts of protein dissolved were too small to enable accurate estimations to be made, but in both cases the crude gliadin, as calculated from the rotation, was not 60 per cent. of that obtained by multiplying the nitrogen by 5.7.

What other substances beside gliadin are dissolved by the phenol has not been determined. No careful experiments in this direction have been made, but the common animal proteins do not seem to be dissolved by it. Witte's peptone dissolves readily. The extremely weak acid character of phenol (its dissociation constant being not more than one two-hundredth that of carbonic acid) hardly leads one to suspect that it acts similarly to the strong acids on the other wheat proteins. In fact an alcoholic solution of phenol has been advocated as a quantitative protein precipitant.[1]

It might seem likely that besides the gliadin but one other wheat protein dissolves in phenol. Amides and proteoses are present in very small amount and it is doubtful if the former are soluble. If x represents the true percentage of gliadin in a flour and but two proteins are present in the phenol solution, then the specific rotation of the second protein in phenol would be:

$$[\alpha]_D^{40} = \frac{(\text{crude gliadin calculated from rotation of extract}-x)}{(\text{crude gliadin calculated from nitrogen of extract})-x} \cdot 132$$

regardless of whether the extraction of the second protein were complete or not. The great influence of small experimental errors on this ratio renders it of comparatively little value, but in no case do the percentages of gliadin by a given method, when substituted for x, give concordant values for $[\alpha]$ for the four samples.

¹ Jago, Science and Art of Bread-making, p. 585.

Air-dry flour treated with phenol yields mixtures so difficult to filter that they were not investigated. Single extractions made with samples 2 and 3 gave the following values which, though not accurate, show that the protein dissolved from the air-dry flour is not pure gliadin.

No. of sample.	Crude gliadin calculated from nitrogen determination.	Crude gliadin calculated from polariscope reading.
2	5.24	4.15
3	7.80	6.43

The filtration of the extract was so slow that it may have changed more or less in concentration from evaporation or absorption of water.

Summary.

With these flours 8 to 17 per cent. more nitrogenous matter was extracted when 4 grams per 100 cc. of the solvent was used than when four times as much flour was taken.

After drying six hours in the water oven, 10 to 20 per cent. less gliadin was obtained by extracting with cold solvent. With the hot solvent the figures were nearly the same, being slightly lower. Pure gliadin remains soluble in dilute alcohol after the same treatment.

No tendency for glutenin to remove gliadin from its alcoholic solutions by absorption or with the production of a solid solution could be demonstrated.

Propyl alcohol of constant boiling-piont (70 per cent. by weight) used in an extraction apparatus gave results probably no more accurate than the others.

Anhydrous phenol dissolves a high percentage of protein matter from the flour. The dissolved matter is not pure gliadin, however, nor does it seem to consist of gliadin with but one other protein.

I acknowledge with pleasure and gratitude the encouragement I have enjoyed from Prof. J. T. Willard in making these experiments, also my indebtedness to Mr. C. O. Swanson for much valuable data concerning the samples.

KANSAS STATE AGRICULTURAL COLLEGE,
October 18, 1907.

THE EFFECT OF NITROGEN PEROXIDE UPON WHEAT FLOUR.

BY F. J. ALWAY AND R. M. PINCKNEY.

Studies on the bleaching of flour by means of the oxides of nitrogen have been published by Avery,[1] Ladd,[2] and Snyder[3] in this country, by

[1] This Journal, 29, 571 (1907).
[2] Bull. 72, N. D. Agr. Exp. Sta. (1906).
[3] Report on bleaching of flour.