CRUDE GLUTEN.

By F. A. Norton.

Received October 27, 1905.

The necessity of determining the gluten content in the valuation of wheats and flours is well recognized and among the different methods employed, the determination as crude gluten has been the one most often used. In this article will be considered the composition of crude gluten, the relation of the crude gluten content to that of total protein in wheat and flour and crude gluten in the valuation of wheats and flours, and in comparison with other methods of determining the content and quality of the gluten.

THE COMPOSITION OF CRUDE GLUTEN.

Crude gluten, the insoluble proteids of wheat together with more or less adhering impurities from the non-proteid constituents of the wheat, is readily obtained by kneading a dough from wheat flour or meal in a stream of water until the wash-water is free from turbidity. Thorpe[1] gives the approximate composition of fresh crude gluten as consisting, besides water, of a certain amount of starch, fat, other débris and proteid substances differently named by various authors. Leach[2] states that crude gluten is a complex mixture of many bodies, containing besides gliadin and glutenin, small quantities of cellulose, mineral matter, lecithin and starch. Allen[3] gives much the same composition for crude gluten as Leach, stating that it consists of a mixture of proteids, principally gliadin and glutenin, together with small quantities of lecithin, fat, phytosterol, starch, cellulose, fibre and mineral matter. There has been much work done on the proteids of wheat with great discordance in results but the findings of the thorough investigation of wheat proteids by Osborne and Voorhees[4] are now generally accepted as correct. In this treatise they have shown very conclusively that gluten consists essentially of two proteids—gliadin,[5] soluble in 70 per cent.

[1] "Dictionary of Applied Chemistry," Vol. I, p. 33.

[2] "Food Inspection and Analysis," p. 230.

[3] "Commercial Organic Analysis," Vol. IV, p. 78.

[4] Am. Ch. J. 15, 392.

[5] Ritthausen and some continental chemists hold that there are three distinct proteids—glutin fibrin, gliadin and mucedin—soluble in 60 to 70 per cent. alcohol, but Osborne in a recent (Twenty-eighth Ann. Rep. Conn.

alcohol and glutenin, soluble in dilute potassium hydroxide solution—which exist as such in the wheat and which unite when moistened to form the gluten. The glutenin imparts solidity to the gluten by forming a nucleus to which the gliadin adheres, while the gliadin forms a sticky medium binding together the particles of the flour, thus rendering the dough tough and coherent. The authors quoted have evidently based their statement of the composition of crude gluten quite largely upon the work of Osborne and Voorhees with which it is in agreement.

Little can be found as to the amount of fibre, starch and other non-proteid substances in crude gluten. Wanklyn and Cooper,[1] however, state that crude gluten contains ash equal to 0.3 per cent. and fat equal to 1.0 per cent. of the flour. And Chamberlain,[2] on washing out the crude gluten in a slow stream of 1 per cent. sodium chloride solution from two straight durum wheat flours and one Kansas hard winter wheat flour, found an average nitrogen content of 13.19 per cent. for the glutens. This treatment, which should have removed all proteids except gliadin and glutenin, gives 75.14 per cent. (taking $N \times 5.7$) of true gluten in the crude gluten.

In order to determine more fully, the proteid and non-proteid substances present in crude gluten, the following proximate analysis was made on the crude gluten from a sample of mixed straight durum wheat flours, as milled in this laboratory. The flours, six in number, after thorough mixing were sampled and crude gluten and total nitrogen determined on the sample. Nitrogen was determined by the Gunning method and crude gluten by washing as directed by Wiley,[3] the gluten being dried to constant weight at 100°. Also a larger amount of crude gluten was prepared by operating on 100 grams of flour. The resulting crude gluten, after being partially dried at 100°, was ground in a mortar until it would pass a 0.5 mm. sieve and then dried until it ceased to lose weight at 100°, when it was placed in weighing-bottles and kept over sulphuric acid until sampled, for analysis. Determinations of ash, ether extract, fibre, carbohydrates other than fibre and protein were then made in duplicate on the sample,

Agr. Expt. Sta. 1904, p. 457) reinvestigation of the alcohol-soluble proteid of wheat finds the results of the former work fully confirmed.

[1] "Bread Analysis," p. 43.

[2] Bull. No. 81, p. 121, Bureau of Chem., U. S. Dept. of Agr.

[3] "Principles and Practice of Agricultural Analysis," Vol. III, p. 435.

according to the methods of the Association of Official Agricultural Chemists.[1] The starch and carbohydrates other than fibre were determined by the acid hydrolysis with determination of the resulting glucose by the Defren-O'Sullivan Method.[2] The factor 0.9 was used for the conversion of glucose to starch and the carbohydrates all returned as starch.[3] The results which follow are referred to both the crude gluten and to the flour.

	Per cent. in crude gluten.	Per cent. recovered in crude gluten from flour.
Total protein ($N \times 5.7$)	80.91	13.39
Ether extract	4.20	0.69
Fiber	2.02	0.34
Ash	2.48	0.41
Carbohydrates other than fiber	9.44	1.56
Total	99.05	16.39
Crude gluten in flour		16.55
Total protein in flour ($N \times 5.7$)		15.25

Proteids in Crude Gluten.—As it was desired to ascertain the character of the proteid contents of the crude gluten, crude gluten was obtained in duplicate, as above on two 5-gram portions of the flour and subjected to the following determinations. After completing the washing of the crude gluten, the samples were finely divided, placed in flasks and extracted with successive portions of 70 per cent. alcohol for twenty-four hours, 250 cc. of alcohol being used for each sample. The alcohol extracts were transferred to Kjeldahl digestion flasks and after distilling off the alcohol, nitrogen was determined by the Gunning process. The gluten residues were then extracted with successive portions of 10 per cent. sodium chloride solution for six hours, 250 cc. of solution being used for each sample and nitrogen determined on the extract after concentration with a little sulphuric acid. The gluten residues were then extracted with 250 cc. of 0.2 per cent. potassium hydroxide solution for six hours. Practically all the residue went into solution but a small amount remained,

[1] Bull. No. 46, Bureau of Chem., U. S. Dept. of Agr.

[2] This Journal, 18, 749.

[3] Osborne has shown (This Journal, 25, 474) that while a carbohydrate group is present in the protein molecule of some proteids, the amount present is extremely small, so that the method employed should give closely the non-fibre carbohydrates retained in the crude gluten. Besides the starch which could be identified microscopically in the crude gluten the hydrolysis would probably remove some pentosans and hemi-cellulose.

which appeared to be largely fibre. After filtering off and washing the residue, nitrogen was determined in the extract as with the sodium chloride extract. Results follow expressed on the flour:

	Nitrogen in gluten from 5 grams of flour. Grams.	Per cent. of proteid (N × 5.7) recovered in the gluten from the flour.
Gliadin or 70 per cent. alcohol extract	0.0564	6.42
Glutenin or 0.2 per cent. KOH extract	0.0505	5.76
Globulin or 10 per cent. NaCl extract	0.0055	1.11
Total proteids recovered		13.29
Total protein recovered in gluten from flour		13.39

This gives practically the same amount of protein recovered in the several extracts as by direct determination of total protein on the dry crude gluten and together with the non-proteid constituents would give the following proximate composition for the crude gluten.

	Per cent.
Fats or ether extract	4.20
Carbohydrates other than fiber	9.44
Fiber	2.02
Ash	2.48
Gliadin	39.09
Glutenin	35.07
NaCl soluble protein	6.75
Total	99.05

This gives 74.16 of gliadin and glutenin or true gluten in the crude gluten and compares very closely with Chamberlain's figures, which indicate 75.14 per cent. proteid in the crude gluten, all of which should have been true gluten. Determinations of protein on the dry crude gluten from a patent spring wheat flour, a patent durum wheat flour, a whole durum wheat meal together with the above sample gave the following results:

	Per cent. protein (N × 5.7) in crude gluten.
Straight durum wheat flour	80.91
Patent durum wheat flour	77.86
Whole durum wheat meal	77.52
Patent spring wheat flour	82.74

These show quite close agreement in the protein content and it is probable that the glutens would correspond quite closely in other respects. This would indicate that crude gluten contains approximately 75 per cent. of true gluten, together with variable amounts of fat, carbohydrates, mineral matter and non-gluten proteid matter.

RELATION OF CRUDE GLUTEN TO TOTAL PROTEIN, $N\times5.7$.

There is quite a close agreement between the crude or dry gluten and the total proteid ($N\times5.7$) content of flour. R. Heinrich[1] states that the gluten content of wheat generally but not always rises and falls with the nitrogen content. In "Cereals and Cereal Products"[2] the authors, in discussing dry gluten, state that in general there is a common agreement in the flours analyzed, between the total percentage of dry gluten and of total proteid matter; that those flours which show a high content of total proteid matter also show a high content of dry gluten. The Columbus Laboratories in their report[3] to the Tennessee Experiment Station on the "Relative Values of Varieties of Winter Wheat for the Production of Flour and Bread" have taken $N\times5.7$ as gluten, stating: "This was found to be more constant and reliable for work of this character than the determination of crude gluten by hand washing. In general the amounts of gluten in white flours as determined by the two methods are found to agree closely."

A great many dry gluten determinations made by the writer, in a general way, confirm the work of those quoted. However, there is a variation of from about 2 per cent. less dry gluten than protein, in some cases, with whole wheat meal to about 4 per cent. more dry gluten than protein with some straight durum wheat flours. In general, a straight or baker's grade flour gives a higher average crude gluten content in relation to the protein content than a patent flour, while a patent flour in turn gives a higher crude gluten content in relation to the protein content than a whole wheat meal.

The following determinations of dry or crude gluten collated from the results of a number of workers give the general relation between crude gluten and total protein and show that only the crude gluten of patent flours approximates very closely to the total protein content, while that of straight or baker's grade

[1] Zweiter Ber. landw. Ver. Stat. Rostock, 1894, pp. 213–223.

[2] Bull. No. 13, Part 9, p. 1259, Bureau of Chem., U. S. Dept. of Agr.

[3] Bull. of Tenn. Agr. Expt. Sta., Vol. XVI, No. 4, p. 57.

Flour.	No. of determinations.	Protein N × 5.7.	Dry gluten.	Difference.	By whom made.
Straight spring wheat, without low grade........	10	11.06	12.68	+1.62	Central Expt. Farm, Canada.[1]
Patent durum wheat.......................................	10	12.61	14.13	+1.52	Chamberlain, Bureau of Plant Ind.[2]
Patent northwest spring wheat	6	13.01	13.53	+0.52	" " " " "
Patent Kansas hard winter wheat.....................	1	12.85	12.64	—0.21	" " " " "
Straight Kansas hard winter wheat.................	3	14.69	15.92	+1.23	" " " " "
Straight northwest spring wheat	3	13.39	15.15	+1.76	" " " " "
Straight durum wheat	3	15.65	17.96	+2.31	" " " " "
Whole wheat meal, all kinds..........................	228	11.13	10.22	—0.91	T. C. Trescot, Bureau of Chemistry.[3]
Whole wheat meal..	6	13.04	11.72	—1.32	Maine Expt. Station.[4]
Patent, spring and winter................................	4	11.96	11.85	—0.11	Minn. Expt. Station.[5]
Patent...	40	9.62	9.99	+0.37	Bureau of Chemistry.[6]
Common market..	19	9.28	9.21	—0.07	" " "
Baker's grade..	14	11.20	13.07	+1.87	" " "
Straight durum, low grade included............. .	113	14.37	16.59	+2.22	Norton, South Dakota Expt. Station.[7]
Straight spring wheat, low grade included	5	12.66	14.82	+2.16	" " " " "
Patent durum	1	10.03	10.91	+0.88	" " " " "
Patent spring wheat....	1	10.64	10.35	—0.29	" " " " "
Whole durum wheat meal...........	1	14.24	12.94	—1.30	" " " " "

[1] "Milling and Chemical Value of the Grades of Wheat," Bull. 50, Part II.
[2] "Commercial Status of Durum Wheat," Bull. 70, Bur. of Plant Ind., Dept. of Agr.
[3] "Analyses of Cereals Collected at the World's Columbian Exposition," Bull. 45, Bureau of Chem., U. S. Dept. of Agr.
[4] "Wheats and Flours of Aroostook County," Bull. 97, Maine Expt. Sta.
[5] "Wheat and Flour Investigations," Bull. 85, Minn. Expt. Sta.
[6] "Cereals and Cereal Products," Bull. 13, Part 9.
[7] "Macaroni Wheat," Bulls. 82 and 92, So. Dak. Expt. Sta.

flour and whole wheat meal often varies several per cent. from the total protein.

In order to account for the variation in the relation of the crude gluten content to that of total protein with the different flours we will need to consider the character both of the proteids and of the non-proteid constituents. The only non-proteid which might be very largely responsible for this variation is the fibre. The writer has found the crude gluten to be very tenacious in retaining the fibre and in the analysis of crude gluten given above it is seen that the fibre recovered in the crude gluten is equal to the average fibre content for a straight grade flour. This has also been the experience of Lindet and Amman who state[1] in a discussion of the "Influence of Some Constituents of Whole Meal Flour on the Extraction of Gluten" that the gluten extracted from flour containing a small amount of bran is usually found to contain less nitrogen than is present in ordinary gluten and account for it by the fact that the bran is separated from the flour along with the gluten. As regards the fibre content, we find average amounts for the different grades of flour and whole wheat as follows:

	Number of analyses.	Per cent. fibre.
Patent wheat flours	40	0.21[2]
Baker's and family grade flours	14	0.22[2]
Straight flours	10	0.34[3]
Whole wheat	228	2.35[4]

It is probable that the variation between the crude gluten and protein contents is due more largely to the character of the proteids. Osborne and Voorhees[5] give the proteids of the wheat kernel approximately as follows:

	Per cent.
Globulin	0.6–0.7
Albumin	0.3–0.4
Proteose	0.3
Gliadin	4.25
Glutenin	4.0–4.5

This would give of the total proteids about 86 or 87 per cent.

[1] C. R. 141, 56–58 (1905).

[2] Bull. No. 13, Part 9, Bureau of Chem., U. S. Dept. of Agr.

[3] Bull. No. 50, Part II, Central Expt. Farm, Canada.

[4] Bull. No. 45, Bureau of Chem., U. S. Dept. of Agr.

[5] Am. Ch. J. 15, 468.

of gliadin and glutenin or true gluten. Snyder[1] has shown that the germ, bran and shorts of wheat and low-grade flour contain from two to four times more water and salt-soluble proteids than patent flours and states: "The process of milling results partly in a mechanical separation of the various proteids, the non-gluten proteids being recovered mainly in the by-products while the gluten proteids are recovered mainly in the flour. In the wheat, from 80 to 85 per cent. of the total nitrogen is in the form of gluten while in the flour there is from 86 to 89 per cent. in this form."

Of the wheat proteids, the gliadin and glutenin unite to form the gluten and should be recovered in the crude gluten. The proteose and albumen are water-soluble and would be lost in the washing. The globulin is only soluble in salt solutions and would probably be very incompletely removed, as its separation would be mechanical, as with starch and fibre. In addition to these proteids, the nitrogen of wheat consists also of amide nitrogen. Investigators[2] have isolated the amide compounds principally from the germ of the wheat and determinations by different workers indicate that the offal products in the milling of the wheat contain most of the amide bodies, patent flour being practically free. The amides are readily water-soluble and would be lost in washing out the gluten. In order to determine more fully the relative amounts of the proteid and amide bodies in whole wheat and the different flours, the following determinations were made on the same samples as referred to in the forepart of this article. The methods employed were those outlined by Snyder[3] and based upon the work of Osborne and Voorhees. Results follow together with determinations by Snyder on two patent flours and one baker's grade flour.

	Total nitrogen. Per cent.	Water soluble nitrogen. Per cent.	NaCl soluble nitrogen. Per cent.
Straight durum wheat flour	2.68	0.46	0.33
Patent spring wheat flour	1.87	0.38	0.15
Whole durum wheat meal	2.50	0.49	0.34
Average of two patent spring wheat flours	2.24	0.42	0.20
Baker's grade flour[4]	3.28	0.38	0.30

[1] Ann. Rep. Minn. Expt. Sta., 1899, p. 529.

[2] Bull. No. 13, Part 9, p. 1207.

[3] Ann. Rep. Minn. Expt. Sta., 1899, p. 524: also Bull. 81, p. 103, Bureau of Chem., U. S. Dept. of Agr.

[4] Ann. Rep. Minn. Expt. Sta., 1899, p. 524.

The water-soluble gliadin nitrogen and the amide nitrogen were then determined and the non-gluten nitrogen calculated by subtracting the water-soluble gliadin nitrogen from the water-soluble nitrogen and adding the sodium chloride-soluble nitrogen to the latter.

	Amide nitrogen. Per cent.	Water-soluble gliadin nitrogen. Per cent.	Nitrogen of albumen and proteose. Per cent.	Non-gluten nitrogen of total nitrogen. Per cent.
Straight durum wheat flour.....	0.04	0.29	0.13	18.65
Patent spring wheat flour........	0.02	0.25	0.11	14.97
Whole durum wheat meal	0.09	0.24	0.16	23.60
Patent spring wheat flours— average of two	0.016	0.34	0.08	13.80
Baker's grade flour................	0.024	0.29	0.09	12.93

From the above we can see that the best flours contain more true gluten but considerably less sodium chloride-soluble proteid which might be retained in the washing out of the crude gluten. Also that there would be more loss from amide nitrogen and water-soluble proteids with the whole wheat and low-grade flours than with patent flours.

Since the washing out of the crude gluten is a comparatively rough process, we have now to consider what loss of true gluten might take place during the operation. Osborne and Voorhees,[1] in their work on wheat proteids, report that they obtained the same amount of gliadin by direct extraction of the whole wheat meal with 70 per cent. alcohol as by the extraction of the crude gluten from an equal amount of the meal. Snyder,[2] however, found that direct extraction of flour with alcohol for the determination of gliadin gave better results and offered fewer difficulties than the extraction of the gluten mass from a like quantity of flour. In the flours previously described we have the following percentages of the total protein of the flour recovered in the crude gluten. Determinations on a spring wheat and a winter wheat meal by Osborne and Voorhees are included.

[1] Am. Ch. J. 15, 440, 441.

[2] Ann. Rep. Minn. Expt. Sta., 1899, p. 531.

	Protein (N × 5.7) in flour. Per cent.	Dry gluten in flour. Per cent.	Protein in crude gluten. Per cent.	Total protein of flour recovered in crude gluten. Per cent.
Straight durum wheat flour	15.25	16.55	80.91	87.80
Patent spring wheat flour	10.64	10.35	82.74	80.49
Patent durum wheat flour	10.03	10.91	77.86	84.69
Whole durum wheat meal	14.24	12.94	77.52	70.44
Whole spring wheat meal[1]	11.93	12.69	68.46	72.82
Whole winter wheat meal[1].	10.96	11.86	68.40	74.02

The above table shows, as the character of the proteids indicated, that more of the total nitrogen is recovered in the crude gluten of flours than in the crude gluten of whole wheat meals. In regard to the relation of total protein recovered to the true gluten content of the several samples we also find variations. In the case of the straight durum wheat flour, we have, by subtracting the non-gluten nitrogen, 81.35 per cent. of true gluten while 87.80 per cent. of the total protein of the flour was recovered in the crude gluten. In the determinations of the proteids in the crude gluten from this flour as given above we found the following percentages for the proteid bodies present.

	Total proteids in crude gluten. Per cent.
Gliadin	48.31
Glutenin	43.35
NaCl soluble in proteid	8.34

This would give 91.66 per cent. of the proteids of the crude gluten as true gluten and 80.47 per cent. of true gluten recovered from the flour as against the true gluten content of 81.35 per cent., which would indicate some loss of true gluten. With the patent flours and whole wheat meal there was less total protein recovered from the flour than the true gluten contents so there must have been considerable loss of true gluten. The two determinations by Osborne and Voorhees show about the same amount of proteids recovered in the crude gluten as in the determination by the writer on whole wheat meal. Osborne and Voorhees[1] give the following proteid composition for the two wheats.

[1] Am. Ch. J. 15, 461.

	Spring wheat.		Winter wheat.	
	Nitrogen.	Proteid.	Nitrogen.	Proteid.
Glutenin	0.8246 × 5.68	= 4.683	0.7346 × 5.68	= 4.173
Gliadin	0.6977 × 5.68	= 3.963	0.6884 × 5.68	= 3.910
Globulin	0.1148	= 0.624	0.1148	= 0.625
Coagulum	0.0453	= 0.269	0.0379	= 0.223
Albumin	0.0657	= 0.391	0.0603	= 0.359
Proteose	0.0341	= 0.213	0.0791	= 0.432
From H_2O washings of gluten	0.2239 × 5.68	= 1.272	0.1552 × 5.68	= 0.881
Total,	2.0050	11.415	1.8703	10.603
Flour	2.10 × 5.68	= 11.93	1.94 × 5.68	= 10.96

This gives for the two wheats the following percentages of gluten and non-gluten nitrogen in the total nitrogen.

	Spring wheat.	Winter wheat.
True gluten	72.64	73.35
Non-gluten	12.55	15.06
H_2O washings	10.66	8.00
Difference between total and proteid nitrogen, probably amide nitrogen	4.15	3.39

As Osborne and Voorhees determined the gliadin and glutenin in the above analysis on the crude gluten obtained by washing a definite amount of the whole wheat meal, any loss of true gluten would be found in the aqueous washings, and it is probable that the true gluten content of the two wheats was somewhat higher than the figures for gliadin and glutenin would indicate. Nevertheless, their figures as to proteids recovered in the crude gluten agree in general with those of the writer.

As to the loss of true gluten, this seems to be greatest in the patent flours and whole wheat meals and must be due to the impairment of the agglutinating power. Macaroni manufacturers object to the flour or semolina for the manufacture of macaroni being finely ground, as in so doing it loses what is called its "force"[1] *i. e.*, its agglutinating power is impaired and this may account for the greater loss of true gluten in the patent flour. In patent flours the smaller sodium chloride-soluble proteid content would also lower the amount of proteids recovered in the crude gluten from the flour. With the whole wheat meal, the large fibre content and perhaps some other

[1] "Manufacture of Macaroni and Semolina," Bull. 20, p. 25, Bureau of Plant Industry, U. S. Dept. of Agr.

factors would effect the agglutinating power of the gluten. Lindet and Amman[1] state that while the gluten of white flour is readily separated by washing and kneading the cake of dough under a stream of water, in the case of whole meal flours or mixtures of the same with white flour a coherent mass of gluten cannot be obtained and ascribe this lack of agglutinating power to the greater acidity of the whole meal flour, to the presence of a mucilaginous substance and greater glutenin content with smaller gluten content, and to the effect of the bran in preventing agglutination. The writer has had no great difficulty in securing good duplicate results in determining the crude gluten on whole wheat meal. Other workers in this country have made a great many determinations of crude gluten on whole wheats, yet it is quite certain that the bran, at least, more or less affects the agglutinating power of the gluten of whole wheat meal.

From the above discussion it will be seen that all of the nitrogen of the flour is not recovered in the crude gluten and thus the relation of dry gluten to total protein in a flour is the balance between the loss of proteid and amide bodies and gain by retention of fibre, starch and other non-proteid matters. The crude gluten from whole wheat flours is the least pure, more non-proteid matter being retained but the greater loss of nitrogenous bodies due to the larger content in the flour of amide compounds and non-gluten proteids, together with greater loss of true gluten, brings the crude gluten content below that of the total protein content. In patent flours there are less non-proteid substances retained in the crude gluten and probably also less of the sodium chloride-soluble proteid which, together with some loss of true gluten, brings the total proteid and crude gluten content nearly the same. In straight and low-grade flours a somewhat larger content of fibre and sodium chloride-soluble proteid together with probably less loss of true gluten especially in coarser flours brings the crude gluten content considerably above that of the total protein.

GLUTEN IN THE VALUATION OF WHEATS AND FLOURS.

Most investigators of wheat and flour determine the crude gluten as a means of ascertaining the bread value of the flour. Some workers determine only the dry crude gluten, others only

[1] C. R. 141, 56–58 (1905).

the moist or wet gluten, while others determine both and express the relation of the two as water capacity.

The moist gluten is usually determined by making 10 grams of flour into a stiff dough, and after allowing it to stand for an hour for the gluten to form, the dough is washed in a stream of cold water until the wash-water is free from turbidity. After standing another hour, the gluten is removed from the dish and the adhering water removed as completely as possible by kneading the gluten mass between the fingers or the moist gluten is permitted to air-dry for a time, when it is weighed and the weight recorded as moist gluten. Dry gluten is determined by drying the moist gluten to constant weight at 100°. The water content or capacity of the gluten is expressed either as the ratio of dry to moist gluten or as the water held by each gram of dry gluten. There are then really two determinations, that of crude gluten either dry or moist and water capacity of the gluten.

The Water Capacity of Glutens.—The reason given for this determination is that a well hydrated gluten lends itself more readily to the fabrication of a loaf of good porosity, sponginess and weight, *i. e.*, a high water content tends to a greater bread yield. There is considerable variation in the water content of glutens as may be seen from the following determinations collated from the reports of a number of workers.[1]

	No. of determinations.	Water held by each gram dry gluten. Grams.
Patent flours	40	1.60[2]
Baker's grade flours	14	1.65[2]
Straight spring wheat flours with low-grade flour discarded	10	1.86[3]
Straight spring wheat flours with low-grade flour included	5	2.10[4]
Straight durum wheat flours	113	1.96[4]
Patent durum wheat flours	3	1.85[4]

In these determinations we see a general tendency for the better flours to have a low water content. However, results seem to be far from consistent, for Shutt[5] reports practically

[1] The references to the literature containing the above determinations may be found in connection with the determinations of crude gluten cited.

[2] Made by Bureau of Chemistry.

[3] Made by Central Experiment Farm, Canada.

[4] Made by South Dakota Experiment Station.

[5] Bull. 50, Part II, p. 17, Central Experiment Station, Canada.

the same water content for the crude gluten for flours from No. 1 hard and No. 1 Northern wheats as for the flour from wheats graded "feed" and No. 5 frosted. The writer, in a great many determinations on straight durum wheat flours, has found very contradictory results, as sometimes the gluten of the best flours and sometimes that of the poorer ones gave the highest water content. In general, as seen with the patent flours, there seems to be a tendency for the best flours as regards bread purposes to have a low water content. The explanation of this would seem to lie in the higher glutenin content of the gluten of low-grade flours. H. A. Guthrie[1] states that the strength or water-absorbing capacity of a flour depends directly upon the relative proportions in which gliadin and glutenin are present in the gluten and that if the gluten content is nearly the same for two flours that will be the stronger flour which contains the larger proportion of glutenin. Fleurent[2] states that bread made with a flour whose gluten contains as little as 20 per cent. of glutenin rises well during fermentation but flattens in baking. One always uses too much water with such a flour. When the gluten approaches 34 per cent. of glutenin the dough neither rises during fermentation nor in the oven and the bread remains heavy and indigestible. The character of the dough in the latter case cited by Fluerent would indicate too large a water absorption. The experience of the writer agrees with that of those quoted. The following example from our work on durum wheat flours, while characteristic of many other samples, is especially striking. The flour had a high crude gluten content—17.45 per cent. dry gluten—with a water capacity for the gluten slightly above the average and a high water absorption capacity for the flour. The flour yielded a dough which failed to give a good sponge or to rise well during baking, and the bread was heavy, dark, or of very poor texture and the loaf was only about three-fourths the size of the usual loaf from the same amount of flour. The gliadin content of this flour was abnormally low, only 35.74 per cent. of the total protein, which would indicate a decided excess of glutenin. If a gluten has a good water capacity with proper physical qualities, it would be desirable, but there does not seem to be anything very definite about the values obtained and often a high water

[1] Agl. Gaz. N. S. Wales, 1896, No. 9, pp. 583-590.

[2] Manuel l'analyse Chimique, 1898, pp. 308-314.

content goes with excess of glutenin and poor bread-making properties, so that the determination does not seem to be of much value.

Crude Gluten in the Valuation of Wheats and Flours.—The crude gluten is reported sometimes as dry and sometimes as moist gluten. Unless it is desired to report the moist gluten in addition to the dry, it would seem much better to report it in the latter form as the personal equation enters largely into the determinations of moist gluten and even on duplicate samples it is difficult for the same person to get strictly concordant results. The writer, while finding it comparatively easy to get two duplicate samples of dry gluten to agree within 0.1 to 0.2 per cent., has sometimes had the same duplicates vary from 0.5 to 1.0 per cent. on the moist gluten determination.

As to the value of crude gluten as an indication of the value of a flour for bread-making purposes, there certainly is no direct relation between the amount of crude gluten and the volume and texture of the loaf. Snyder,[1] in discussing the bread-making values of roller mill products, states: "a high gluten content does not necessarily imply a large loaf or good bread-making qualities. The sample with the largest gluten content was the fourth break flour which produced a rather small loaf, and one of poor quality. A high gluten content is desirable, provided the gluten is of good quality." The following determinations by the writer on spring and durum wheat flours show how little the volume and the quality of the bread depends upon the crude gluten content of the flour. Results are averages for the flours taken.

	Dry gluten. Per cent.	Volume of loaf. cc.	Weight of loaf. Grams.	Rank as to texture and quality.
Five best straight durum wheat flours...	16.94	1580	455	2
Five poorest straight durum wheat flours	17.12	1290	450	3
Five straight spring wheat flours.........	14.82	1630	447	2
Two patent durum wheat flours............	13.38	1650	430	1
One patent spring wheat flour..............	10.35	1700	435	1

While the crude gluten content is not a criterion of the bread-making value of the flour, yet some idea of the quality of the gluten can be obtained from the crude gluten and a general impression of the value of the flour for bread purposes can be gained. Thus if the gluten is separated with difficulty on washing and is

[1] Bull. No. 85, p. 197, Minn. Expt. Sta.

short or lacking in elasticity, a deficiency of gliadin is indicated and the flour will not make good bread. On the other hand, if the separated gluten lacks body, *i. e.*, spreads out very readily when made into a ball and is over-sticky, an excess of gliadin is indicated and the flour will be equally poor for bread purposes. Nevertheless, no very close values as to the bread-making properties of a flour can be obtained directly from the crude gluten.

The baker's sponge test[1] in connection with the determination of crude gluten gives values which are very indicative of the character of the gluten, as may be seen from determinations on the flours just cited.

	Dry gluten. Per cent.	Average rise for 100 grams flour. cc.	Average rise for each gram dry gluten. cc.	Volume of loaf. cc.
Five best straight durum wheat flours	16.94	670	39.45	1580
Five poorest straight durum wheat flours	17.12	528	30.84	1290
Five straight spring wheat flours	14.82	652	44.00	1630
Two patent durum wheat flours	13.38	650	48.60	1650
One patent spring wheat flour	10.35	720	69.56	1700

However, if a flour gives a good sponge test, that is, an expansion of 650 to 700 cc. for the dough from 100 grams of flour, the experience has been that it will have good bread values, no matter what the crude gluten content may be.

Determination of gliadin might be made on the crude gluten to ascertain whether gliadin and glutenin were present in proper proportions, but it would be more satisfactory to make the determinations on the flour and compare the gliadin content with that of total protein, as closer values could thus be obtained.

It would thus seem that the determination of crude gluten is of questionable value in the valuation of flours and wheats. Snyder, who has been very active in the investigation of flours and wheats, would seem to be of the same opinion. In a recent publication[2] on the testing of wheat flour for commerical purposes he states: "The determinations which have given the best satisfaction in flour testing are moisture, ash, total nitrogen, gliadin nitrogen, granulation, absorptive capacity and color." The same con-

[1] This Journal, 27, 929.

[2] Ibid. 27, 1069.

clusion has been reached by M. Aprin[1] who, after a study of the errors involved in estimating moist gluten, found the method so subject to error as to be entirely unsatisfactory, and he recommends the abandoning of the determination of moist gluten and falling back on the nitrogen content of the flour as a means of judging of its gluten content.

Other Methods of Determining the Gluten Content of Flours.—Among the chemical methods proposed for determining the gluten of flour is that of Fleurent[2] which has been modified by Mangent.[3] In this method the crude gluten is obtained by washing a dough in dilute sodium chloride solution. The separated gluten, after completing the washing, is finely divided and extracted with alcoholic potassium hydroxide solution and gliadin and gluten determined on the extract. The method seems somewhat tedious and results obtained by Chamberlain[4] in a study of the method would indicate that the results obtained are too high. Methods for the determination of wheat proteids, as outlined by Snyder and previously referred to in this article, give good results for the determination of gliadin and glutenin as also do the methods outlined by Chamberlain[5] and based upon the work of Osborne, Voorhees and Snyder. Teller[6] has also proposed methods which give quite concordant results, though it seems advisable always to determine soluble gliadin in the sodium chloride extract.

A more simple method recently proposed is the determination of total nitrogen and gliadin nitrogen and expression of the ratio of gliadin to total protein ($N \times 5.7$). There are not many gliadin determinations on flours recorded. Snyder,[7] however, has suggested that a flour to possess good bread-making properties should contain about 11 per cent. of total protein ($N \times 5.7$) and that 55 to 65 per cent. should be in the form of gliadin.

The experience of the writer is in accord with the above standard and these determinations, which are very accurate and at the same time comparatively simple and rapid, next to the baker's

[1] Ann. Chim. Analyt. 7, Nos. 9–10–11 (1902).
[2] Manuel l'Analyse Chimique, 1898, pp. 308–314.
[3] Rev. intern. des falsif., 1902, p. 91.
[4] Bull. 81, pp. 118–125, Bureau of Chem., U. S. Dept. of Agr.
[5] Bull. 81, p. 124, Bureau of Chem., U. S. Dept. of Agr.
[6] Ark. Expt. Sta., Bull. 42, p. 96.
[7] This Journal, 26, 266.

sponge test or actual baking tests have been found most indicative in determining the value of a flour for bread purposes.

SUMMARY.

Crude gluten consists of about 75 per cent. of true gluten—gliadin and glutenin—together with small percentages of non-gluten proteid, mineral matter, fat, starch, fibre and other non-proteid matter.

The relation of the crude gluten content to that of total protein ($N \times 5.7$) varies with the character of the flour, the crude gluten content being greater than total protein for straight and low-grade flours, nearly the same for patent flours and less for whole wheat meal.

Crude gluten is an expression, in addition to the true gluten content of a flour, of the balance between the loss of non-gluten proteids and gain from the retention of non-proteid substances. The relation of the crude gluten content to the total protein content can thus be explained by the varying composition of the different flours in respect to nitrogenous compounds and non-proteids.

Crude gluten is a very rough expression of the gluten content of a flour or wheat and the determination has but little worth in the valuation of flours.

The determination of total nitrogen and gliadin nitrogen with expression of the ratio of gliadin to total protein ($N \times 5.7$) seems to be the best simple method at hand for estimating the gluten content and ascertaining the character of the gluten in the valuation of wheats or flours.

CHEMICAL LABORATORY,
SOUTH DAKOTA EXPERIMENT STATION.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.]

THE CHEMISTRY OF FLESH.

(THIRD PAPER.)[1]

A STUDY OF THE PHOSPHORUS CONTENT OF FLESH.

BY A. D. EMMETT AND H. S. GRINDLEY.

Received November 13, 1905.

IN CONNECTION with the researches upon the chemistry of flesh which are being made in this laboratory, it seemed desirable

[1] This Journal, 26, 1086 (1904); 27, 658 (1905).