PROTEIDS OF THE WHEAT KERNEL.[1]

By Thomas B. Osborne and Clark L. Voorhees.

THE importance of wheat as a breadstuff, and the fact that its superiority over all other cereals for bread-making mainly depends on the properties of the proteids composing the gluten which wheat dough yields when cautiously washed with water, justify giving here in some detail an account of the present state of knowledge regarding the proteids of wheat.

The wheat used in our work was of two kinds. One of these, Scotch Fife, a hard spring wheat, raised in Minnesota, was obtained through the kindness of Dr. D. N. Harper, late chemist of the Minnesota Experiment Station. It was carefully selected, free from all other varieties, and was milled under the supervision of Dr. Harper, who supplied us with samples of the various mill products, together with some of the unground wheat. Two grades of flour were used; namely, "patent flour," made from the finest and purest middlings, and "straight flour," from the coarser and less pure middlings. The "shorts," chiefly composed of the outer coating of the seeds together with adhering portions of the endosperm, was also examined. Samples of whole wheat flour were prepared as required from the wheat by grinding small quantities in the laboratory mill. The other wheat used was a variety of winter wheat known as "Fultz." This was procured from Mr. F. S. Platt, seedsman, of New Haven, and was carefully selected and freshly harvested.

When wheat flour or meal is made into a stiff dough with water and then carefully kneaded or squeezed in a gentle stream of water, the starch which makes up 60–68 per cent. of the flour or meal is gradually washed away for the most part, and there remains a tough elastic sticky mass.

The first published description of this body was made by Beccari in 1745 who gave it the name it still bears; *viz.*, *Gluten*.

In 1805 Einhof observed that hot alcohol extracts from wheat flour a substance resembling gluten. In 1820 Taddei showed

[1] First printed in the Report for 1893 of the Connecticut Agricultural Experiment Station, New Haven; communicated by the author.

that gluten contains two substances, one soluble the other insoluble in boiling alcohol.

Since 1820 many chemists have undertaken to investigate gluten but their conclusions as regard the number, properties and composition of the proteids it contains, are extremely discordant. Einhof, Boussingault, Bouchardat, Denis, Weyl and Bischoff and Martin have considered gluten to consist essentially of a single proteid. Berzelius, DeSaussure, Liebig, Dumas, and Cahours, and Von Bibra, believed it to contain three, while Ritthausen and Bechamp regard it as composed of four proteids.

We have separated from the wheat kernel five distinct proteids; *viz.*, Gliadin, Glutenin, a Globulin, an Albumin and a Proteose. A proteose-like body, apparently distinct, was also obtained, but in too small quantity for satisfactory examination.

The mode of isolating these substances in a state of comparative purity and establishing their individuality, is fully stated in a former paper,[1] to which reference must be made for details.

Of these proteids only the first two properly belong to gluten.

I. *Gliadin* is the proteid which is readily dissolved from wheat flour and from gluten by hot dilute alcohol. It also exists in the rye-kernel.

This substance when dehydrated by absolute alcohol and thoroughly dried over sulphuric acid, forms a snow-white, friable mass which is easily reduced to a powder. If dried after being moistened with dilute alcohol or water it resembles, in appearance, pure gelatin. Dried thus, in thin sheets it is perfectly clear and transparent but is rather more brittle than gelatin. When treated in the cold with distilled water it becomes sticky and slightly dissolves. If the water is warmed, more dissolves and on boiling, much goes into solution. Solutions in warm water on cooling, deposit a part of the substance. The solution in pure water is instantly precipitated by adding a very minute amount of sodium chloride.

In absolute alcohol gliadin is entirely insoluble, but dissolves on adding water, the solubility increasing with the addition of water up to a certain point, and then diminishing. The exact degree of solubility for various strengths of alcohol has not been

[1] *Am. Chem. J.*, 15, 392.

determined, but mixtures of about seventy per cent. of alcohol and thirty per cent. of water dissolve the proteid in almost indefinite amount. From solutions in strong alcohol as well as from those in very weak alcohol the proteid is precipitated by adding a few drops of solution of sodium chloride, the completeness of precipitation depending on the strength of the alcohol and the amount of salt added. The more the alcohol varies in strength from seventy to eighty per cent. the more completely is the substance precipitated.

In extremely dilute acids and alkalies this proteid is readily soluble, and is precipitated from such solutions on neutralization apparently unchanged either in properties or composition.

Gliadin gives with Millon's reagent, with nitric acid and with the biuret-test, the usual proteid reactions. Dissolved in concentrated hydrochloric acid, a beautiful violet color develops slowly. With warm fifty per cent. sulphuric acid a similar color appears which is greatly increased in intensity on boiling.

On boiling its aqueous solutions, gliadin coagulates and becomes insoluble both in alcohol and in 0.2 per cent. potash solution, but is not thus converted into glutenin, the latter being soluble in weak alkali.

Gliadin was so called by Taddei because of its resemblance to glue. It has also been called plant-gelatin by Liebig, and glutin by Dumas. The plant-casein of Dumas, the mucin of De Saussure and Berzelius, the gluten-fibrin and wheat-mucedin of Ritthausen, are impure gliadin.

Gliadin is entirely distinct in composition from the alcohol soluble proteids of barley, maize, and oats. We found in Fife wheat, 4.33, and in Fultz, 4.25 per cent. of gliadin.

Our analyses of these gluten proteids are as follows:

	Gliadin of wheat, average of twenty-five analyses.	Gliadin of rye, average of thirteen analyses.	Glutenin of wheat, average of eight analyses.
Carbon	52.72	52.75	52.34
Hydrogen	6.86	6.84	6.83
Nitrogen	17.66	17.72	17.49
Sulphur	1.14	1.21	1.08
Oxygen	21.62	21.48	22.26
	100.00	100.00	100.00

II. *Glutenin.* Characteristic reactions of a proteid body which

can be dissolved only in dilute acids or alkalies are necessarily very few in number. Our preparations of glutenin after drying over sulphuric acid, were found to yield to distilled water, especially when warm, a little proteid substance. Diluted alcohol also dissolved a minute amount of proteid matter in the cold, and when heated to boiling, a much greater quantity. It is questionable whether the substance dissolved by water and by alcohol was not a trace of gliadin which had failed to be completely extracted in the process of preparing the glutenin. The fact that the solution in hot alcohol began to precipitate at once on cooling, and that especial care had been taken in every case to remove the gliadin, makes it probable that glutenin is slightly soluble in water and in alcohol, especially if these are warmed.

In very dilute alkalies, as 0.1 per cent. potash solution, and in very dilute acids, as 0.2 per cent. hydrochloric acid, glutenin, after dehydration with absolute alcohol and drying over sulphuric acid, is slowly soluble, with the exception of a greater or less amount of coagulated residue, depending on the circumstances of its preparation. When freshly precipitated, and in the hydrated condition, it is extremely soluble in the slightest excess of caustic alkali, and in a somewhat greater but still very slight excess of acid. In this condition it is also soluble in the slightest excess of sodium carbonate solution or ammonia. After drying over sulphuric acid it dissolves partly in 0.5 per cent. sodium carbonate solution.

Dissolved in concentrated hydrochloric acid it gives a solution slightly yellowish at first, but becoming a deep violet color on standing. In sulphuric acid diluted with an equal volume of water the solution is brownish in color after boiling, and remains clear when diluted. The undiluted solution, on standing, retains its brown color.

A comparison of the analyses of glutenin with those of gliadin shows a very close agreement of the two in composition. As it is well known that many proteids readily lose their solubility without change of composition detectable by analysis, it might seem proper to consider this body as an altered and insoluble gliadin.

There is, however, no evidence that gliadin is actually trans-

formed into glutenin, and since very recent investigation shows that rye-grain contains gliadin, but probably not glutenin, it would appear that the two are to be regarded as distinct.

This proteid, in an impure state, has been termed by various investigators zymom, plant-fibrin, gluten-casein, and gluten-fibrin. As all these names are associated with confused and erroneous notions as to its composition, origin, and properties, they are all properly discarded in favor of the new designation, glutenin.

In Fife wheat we found 3.96, and in Fultz, 3.91 per cent. of glutenin. The other proteids in the wheat kernel are—

III. *Edestin*,[1] a *globulin* belonging to the vegetable vitellins, soluble in saline solutions, precipitated therefrom by dilution, and also by saturation with magnesium sulphate, or ammonium sulphate, but not by saturation with sodium chloride. Partly precipitated by boiling, but not coagulated at temperatures below 100°. The wheat kernel contains between 0.6 and 0.7 per cent. of this globulin. When dried at 110° its composition was found to be as below stated.

IV. *Leucosin*,[2] an *albumin*, coagulating at 52°, unlike animal albumin in being precipitated on saturating its solutions with sodium chloride or magnesium sulphate. It is not precipitated on completely removing salts by dialysis in *distilled* water. It was found to form between 0.3 and 0.4 per cent. of the wheat kernel, and to have the following composition when separated from solution in the coagulated form by heating to 60° C.:

	Edestin.	Leucosin coagulated.	Coagulum from proteose.
Carbon	51.03	53.02	51.86
Hydrogen	6.85	6.84	6.82
Nitrogen	18.39	16.80	17.32
Sulphur	0.69	1.28	24.00
Oxygen	23.04	22.06	
	100.00	100.00	100.00

V. A *proteose*, precipitated (after removing the globulin by dialysis, and the albumin by coagulation) by saturating the solution with sodium chloride, or by adding twenty per cent. of sodium chloride and acidifying with acetic acid. This body was

[1] Ἐδεστὸς edible.

[2] Λευκὸς white.

not analyzed in its unaltered form. On concentrating its solutions by boiling, a *coagulum* was gradually developed which formed about 0.3 per cent. of the wheat kernel and had the composition given above.

VI. The solution filtered from the substance just described (V.) still contained a *proteose-like body* which was not obtainable in a pure state. Its amount could only be roughly estimated by precipitating the concentrated filtrate from the preceding substance with alcohol, and multiplying the nitrogen contained in the precipitate by 6.25. The amount of this proteose was from 0.2 to 0.4 per cent. of the seed. Both this proteose and the above coagulum are unquestionably derivatives of some other proteid in the seed, presumably the proteose first mentioned.

The Formation of Gluten.—Wheat, as far as is known, is the only plant whose seeds contain proteid matter separable in a coherent form from the other constituents by washing with water. When ground fine and mixed with a suitable quantity of water it yields a dough from which a light, porous bread can be made. The importance of this fact in bread-making is so great that considerable attention has been paid to gluten by the chemists who have studied wheat proteids.

The investigations of Günsberg and of Martin, as well as our own, disprove the existence of gluten-fibrin and mucedin, which are currently stated to exist in gluten, and demonstrate that, as Taddei maintained, gluten consists of two proteids only.

Weyl and Bischoff have asserted[1] that the proteid matter of the wheat kernel is *chiefly a globulin substance*, and that in contact with water it undergoes a change, presumably through the influence of a ferment, by which gluten is first produced.

The statements of these investigators are not sustained by any sufficient evidence. They say: "On investigating the proteids of wheat meal, one of us found principally a globulin substance, which he designated, in consequence of its similarity to myosin of the muscle, *vegetable myosin*. This vegetable myosin must be the mother-substance of the gluten, since in the wheat meal, together with it, other proteids, if at all, exist only in very small amount." What the reasons were for concluding that the

[1] *Ber d. chem. Ges.*, *1880*, 367.

"myosin" constitutes nearly, if not all, the proteid of the wheat kernel does not appear. In view of our results this statement is certainly erroneous. Direct treatment of the meal with alcohol yields extracts containing gliadin in exactly the same amount as obtained from the gluten made from an equal quantity of flour, and extraction of either flour or gluten with alcohol, after complete exhaustion with sodium chloride solution, also gives the same proportion of gliadin. This substance must therefore exist in the seed, having the same composition and properties as in the gluten, and as it forms one-half of the gluten, it leaves the other half only as possibly derived from a globulin body through the influence of a ferment. If Weyl and Bischoff's view were correct, treatment of the flour with ten per cent. salt solution ought to alter the character and quantity of the gluten obtained, if not altogether to prevent its formation. This is not so, for the usual amount of gluten can readily be obtained from flour made into dough with ten per cent. sodium chloride solution, and then washed with the same until starch is removed.

Weyl and Bischoff next state that, "with the aid of a fifteen per cent. rock salt solution the flour was extracted until no proteid could be detected in the extract; the residue of the meal kneaded with water then gave no gluten. *If the globulin substance be extracted, no formation of gluten takes place.*" We have found that this is true if the flour is stirred up with a large quantity of salt solution, extracted repeatedly with fresh quantities of the same solution until no more proteid is dissolved, and the excess of solution removed by allowing the residue to drain on a filter as completely as possible. If, however, wheat flour is mixed at first with just sufficient salt solution to make a firm dough, this dough may then be washed indefinitely with salt solution, and will yield gluten as well, and as much, as if washed with water alone. This difference is due to the fact that when large quantities of salt solution are applied at once, the flour fails to unite to a coherent mass and cannot afterwards be brought together, as is possible when treated with smaller quantities of solution.

Weyl and Bischoff compare the formation of gluten to that of blood-fibrin from fibrinogen under the influence of a ferment.

They say that the formation of gluten is affected by all the conditions which interfere with the activity of ferments in general. "Large amounts of salt hinder the formation of gluten. Sulphates of magnesium and sodium behave like common salt." These statements are explained by what has been said above.

They tried unsuccessfully to obtain the supposed ferment in the following manner:

"We allowed meal with an equal weight of ninety per cent. alcohol to stand in closed vessels different lengths of time (in one case four months, then several times three to four weeks, frequently only eight to ten days). The vessels were repeatedly shaken and the yellow-colored alcoholic extract was poured off. The residue was freed from alcohol by pressing and evaporating at the ordinary temperature. After it was stirred up with water little or no gluten was obtained. Evidently the globulin substance had been coagulated for the most part by the alcohol." It is clear that if the flour were thus treated, the greater part of the gliadin would be removed. We have found that if flour be extracted with dilute alcohol until the gliadin is removed, and the residue freed from alcohol by exposure to the air, the latter will then yield no gluten when treated with water.

More recently Sidney Martin has advanced a somewhat similar theory of the formation of gluten from the proteids contained in the seed. He states[1] that alcohol extracts from gluten but one proteid substance; that this is soluble in hot water, but not in cold, and he therefore calls it an *insoluble phytalbumose*.

The residue of the gluten not dissolved by alcohol is uncoagulated proteid, if the alcohol has not been allowed to act too long. This substance he names *gluten-fibrin*. Martin further says that gluten dissolves almost completely in 0.2 per cent. hydrochloric acid, or 0.2 per cent. potash solution, leaving a small residue of fat. The solution gives a copious precipitate when neutralized, but the supernatant liquid still contains a quantity of proteid, which is the dissolved insoluble albumose. The whole of the gluten-fibrin is reprecipitated by neutralization, that is, it is wholly converted into an "albuminate."

Martin then asks: "Does flour contain gluten-fibrin? Does it

[1] *British Medical Journal*, 2, 104, *1886*.

contain insoluble phytalbumose?" The first question he says can not be answered directly. "The second is answered by extracting flour with seventy-six to eighty per cent. alcohol. This ought to contain the insoluble albumose if it were present as such in the flour, *but it does not contain it;* it extracts only fat." This statement is contrary to our experience, for we have never failed, in many experiments, thus to extract this substance (gliadin) from the flour, and that too in the same amount and of the same properties and composition as from the gluten.

Martin concludes that insoluble albumose is not present as such in the flour. He then says: "Before proceeding to mention its precursor, it will be well to state that ten per cent. sodium chloride solution extracts from flour a large quantity of globulin and of albumose. This globulin is of the myosin type, coagulating between 55° and 60° C., and precipitated by saturation with sodium chloride and ammonium sulphate. Both the globulin and albumose are present in a much smaller quantity in the watery extract of the flour." From this it is evident that Martin has fallen into the same error as Weyl and Bischoff, mistaking the albumin for a myosin-like globulin, and being greatly misled as to its amount.

Continuing, Martin says: "The direction of the evidence is to show that the insoluble albumose is formed from the soluble. Moreover, I think that the globulin is transformed into the gluten-fibrin, for I have been able to obtain from the globulin in solution a body having the same reactions as the gluten-fibrin." What this evidence is, which, by its direction, shows that the insoluble albumose is derived from the soluble, is not clear, and Martin makes no further statements on this point. That a body should be obtained from the solution containing the globulin which had the same reactions as the "gluten-fibrin" is not surprising, for the so-called "albuminates" derived from nearly all globulins have no characteristic reactions, being merely soluble in dilute acids and alkalies, and precipitated by neutralization in the same way as "gluten fibrin." Martin then states his theory of the formation of gluten in the following scheme:

$$\text{Gluten} = \begin{cases} \text{Gluten-fibrin} & \text{---precursor, globulin.} \\ \text{Insoluble albumose} & \text{---} \quad \text{soluble albumose.} \end{cases}$$

This can not be a correct representation of the formation of gluten, for it has been shown to be founded on two erroneous observations: First, that alcohol does not extract proteid matter from the flour when applied directly, and second, that at least one-half the proteid matter of the seed is a myosin-like globulin.

The results obtained by us, and described at length in our paper,[1] lead to the conclusion that no ferment action is involved in the formation of gluten; that but two proteid substances are contained in the gluten, gliadin, and glutenin, and that these exist in the wheat kernel in the same form as in the gluten, except that in the latter they are combined with water in an amount equal to about twice the weight of the water-free proteids. The reasons for this opinion are, first, that alcohol extracts the same gliadin in the same amount, whether applied directly to the flour, to the gluten, or to the flour previously extracted with ten per cent. sodium chloride solution; second, that 0.2 per cent. potash extracts glutenin of uniform composition and properties from flour which has been extracted with alcohol or with ten per cent. sodium chloride brine and then with alcohol, as well as from gluten which has been exhausted with alcohol.

Both glutenin and gliadin are necessary for the formation of gluten, as shown by the three following experiments:

1. A portion of flour was washed completely free from gliadin by means of alcohol of 0.90 sp. gr., next with stronger alcohol, finally with absolute alcohol, and air-dried. The residue was then rubbed up fine until all lumps were removed, and water carefully added, and a dough made of the mass. A tolerably coherent dough was thus obtained, but much less elastic and tough than that produced from the untreated flour. This dough was then washed with water on a sieve, using every precaution to obtain a gluten, but none was formed.

2. Again, 7.5 grams of very finely ground air-dried gliadin were mixed intimately with seventy grams of fine corn-starch and distilled water added. A plastic dough was thus produced, but it had no toughness. On adding a little ten per cent. sodium chloride solution, the dough became at once tough and elastic. This was then washed with great care on a sieve with cold water,

[1] *Am. Chem. J.*, 15, 392–471.

a little ten per cent. salt solution being added from time to time, but in spite of every precaution no gluten was obtained.

3. The following experiment shows that the gliadin used was capable of forming gluten when glutenin was present, and also that salts have a marked influence on the toughness of wheaten dough.

Two portions of flour, weighing 100 grams each, were taken, and after adding five grams of gliadin to one, both were made into dough with the same quantity of water. The two doughs presented marked differences; that to which the gliadin had been added was much tougher and more yellow than the other. They were then washed with water as long as starch separated. The gluten was dried superficially by wiping with a cloth and weighed in the moist state. That from 100 grams of flour to which five grams of gliadin had been added weighed 44.55 grams; that from 100 grams of flour alone weighed 27.65 grams. The moist glutens were dried at 110° to constant weight, and both yielded the same proportion of dry gluten; *viz.*, 34.6 per cent. The yield of dry gluten was accordingly, in the first case, 15.41 grams, and in the second, 9.56 grams. The difference, 5.85 grams, shows that the added gliadin was fully recovered in the gluten.

The above figures show that these proteids combine with about twice their weight of water in forming gluten. The fact that the added gliadin entered so readily and completely into the formation of gluten indicates that it exists in the seed as such and undergoes no chemical change during extraction and drying.

The properties observed in testing the separated gliadin show how it acts in forming gluten, and explain many of the points observed by others and attributed to a ferment action.

When treated with distilled water in small amount the fine ground air-dry gliadin at once forms a sticky mass which, on adding more distilled water, dissolves to a turbid solution. If, however, a very little sodium chloride is added to distilled water and this applied to gliadin that has been first moistened with pure water, a very coherent, viscid mass results which adheres to everything it touches, and can be drawn out into long threads. If the gliadin is moistened with ten per cent. sodium chloride

solution and then treated with a larger quantity of this solution, the substance unites to a plastic mass which can be drawn out into sheets and strings, but is not adhesive. From this it is evident why Ritthausen, in washing flours which gave a fluid gluten, obtainable only in small quantity and with great difficulty, found that the addition of calcium sulphate to the wash-water rendered the gluten much more coherent and easily obtainable. The gliadin is thus proved to be the binding material which causes the particles of flour to adhere to one another in forming a dough.

But the gliadin alone is not sufficient to form gluten, for it yields a soft and fluid mass which breaks up entirely on washing with water. The insoluble glutenin is probaby essential by affording a nucleus to which the gliadin adheres and from which it is not mechanically carried away by the wash-water.

It might be supposed that this insoluble glutenin, which so nearly resembles gliadin in composition, results from an alteration of the latter, brought about by the action of the mineral or other constituents of the seed or of the water. This is not probable, for the same amount of gliadin is extracted from flour directly by treating it with alcohol of 0.90 sp. gr., as is obtained from the gluten itself, and also the same amount is obtained after extracting the flour completely with ten per cent. sodium chloride solution, and then with alcohol.

The behavior of the gliadin towards ten per cent. sodium chloride solution shows why no gluten was obtained by Weyl and Bischoff from flour extracted with this solvent. The gliadin had under these conditions no ahhesive qualities, and therefore was unable to bind the flour into a coherent mass. If, however, the salt solution is added in small quantities, and the flour kneaded and pressed, the particles are brought together and then adhere tanaciously.

DEGRAS.[1]

By Charles S. Bush.

IN this country, the term "degras" is generally applied to the grease or fatty matter recovered from the water in which wool has been scoured; in various portions of Europe, the term

[1] Read before the Rhode Island Section, January 25, 1894.