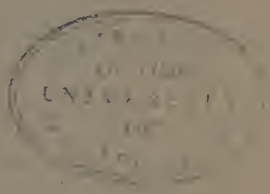
On the Chemical Constitution of the Proteins of Wheat Flour and its Relation to Baking Strength.

Morris J. Blish

On the Chemical Constitution of the Proteins of Wheat Flour and Its Relation to Baking Strength



A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA

BY

MORRIS J. BLISH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

JUNE, 1915

EASTON, PA.:
ESCHENBACH PRINTING CO.
1916

On the Chemical Constitution of the Proteins of Wheat Flour and Its Relation to Baking Strength

---

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA

BY

MORRIS J. BLISH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

JUNE, 1915

EASTON, PA.:
ESCHENBACH PRINTING CO.
1916

ON THE CHEMICAL CONSTITUTION OF THE PROTEINS OF WHEAT FLOUR AND ITS RELATION TO BAKING STRENGTH

By M. J. Blish

Received June 7, 1915

INTRODUCTION

The most generally accepted definition of "baking strength" of a wheat flour is that put forward by Humphries and Biffen,[1] in 1907, which states that a "strong wheat is one which yields flour capable of making large, well-piled loaves;" a definition similar to that of Jago,[2] who states that "strength.....is defined as the measure of the capacity of the flour for producing a bold, large-volumed, well-risen loaf." Since the value of wheat (other things being equal) depends on the so-called "strength" of the flour which may be made from it, it is obviously of great importance that complete knowledge be obtained concerning the factors which cause strength, and to this end an enormous amount of scientific work has been done, especially during the last twenty years. In spite of the fact that some of the foremost investigators of the world have bent their energies to this task, the problem is not yet completely solved, although considerable light has been thrown on the subject. It is not yet possible to correlate baking strength with any chemical or physical factor to such an extent that a simple laboratory test or group of tests will always furnish an infallible guide, but it is necessary to mill the wheat into flour and have a sample of it actually baked into a loaf of bread by an expert baker, before its strength can be accurately ascertained.

FACTORS WHICH MAY INFLUENCE BAKING STRENGTH

Almost every known constituent, or group of constituents, and almost every known physical and chemical property of flour has been investigated with respect to its possible relation to baking strength, but as yet no one is believed to have discovered a limiting factor or group of factors which completely solves the problem. Moreover, there is a general disagreement among many of the leading investigators as to the importance which should be attached to each factor

[1] "The Improvement of English Wheat," *Jour. Agr. Sci.*, **2** (1907), 1–16.

[2] "Technology of Bread Making," Chap. XV, p. 291, 1911.

or set of factors, and two workers frequently have arrived at exactly opposite conclusions after having investigated practically the same problem; however, much of this confusion is caused by the use of different methods of analysis.

A brief review of some of the more important work which has been done will serve to bear out the preceding statement, as well as to indicate the many sides from which the question of flour strength has been studied.

GLIADIN-GLUTENIN RATIO—As soon as Osborne and Voorhees,[1] in 1893, established the composition and properties of the wheat proteins, attention was attracted to gliadin and glutenin, the two conspicuous and characteristic proteins of wheat, which were shown to make up the gluten, the more or less elastic binding material which enables flour to be made into the dough with its characteristic elastic, gas-retaining property, and which may be separated from the starch and soluble proteins by the well-known process of washing the dough in a stream of water. Fleurent,[2] in 1896, claimed that flour strength depends on the proportion of gliadin to glutenin present in the gluten of the flour. He concluded from his experiments that the optimum ratio was 75 parts of gliadin to 25 of glutenin or 3:1. He assigned certain limits, outside of which flours were said to be of poor baking quality. Snyder,[3] in 1899, published similar results, although he fixed his ideal ratio at 65:35. He also states[4] that the quality rather than the quantity of gluten is the important factor, because he was able to add up to 20 per cent starch to flour without decreasing its baking quality. Regarding the quantity of gluten in flour, the amount of gliadin present, the ratio of gliadin to glutenin, and the relation of these to baking quality, it suffices to say that the results of different investigators quite frequently are not concordant. However, as mentioned before, this is in a considerable measure due to different analytical methods employed by different workers.

CRUDE GLUTEN—The crude gluten determination, which consists essentially of washing the gluten free from starch and soluble material by means of water, and weighing the gluten, both in a wet and dry state,

[1] "The Proteids of the Wheat Kernel," *Am. Chem. J.*, **15** (1893), 392–471; *Ibid.*, **16** (1894), 524–535.

[2] "Sur une method chimique d'appreciation de la valeur boulangere des farines de blé," *Compt. rend.*, **123** (1896), 755–758.

[3] *Minn. Exp. Sta. Bull.*, **62** (1899).

[4] U. S. Dept. Agr., *Bull.* **101** (1901).

was for a long time considered of great value, but Snyder and Norton,[1] in 1906, Chamberlain,[2] in 1906, and others showed that it gave but little information which might not be gained from a determination of total nitrogen or alcohol-soluble nitrogen. Nevertheless, it is still used extensively by millers and bakers, and in technical laboratories.

PHYSICAL STATE OF GLUTEN AND SUGAR CONTENT—In 1907, Wood[3] published the results of a thorough and systematic study of the chemistry of flour strength. He concluded that there is no difference in the chemical constitution of gliadin and glutenin from strong and weak flours, and decided that strength (particularly shape of loaf) is much more closely related to the physical state of gluten, which in turn is profoundly affected by the presence of electrolytes. He showed that minute quantities of acids and bases tend to "disperse" gluten, making it weak and inelastic, while small quantities of neutral salts have the opposite and consequently beneficial effect. Furthermore, he found that the volume of a loaf of bread is proportional to the rate of carbon dioxide evolution resulting from diastatic activity of yeast in the later stages of fermentation. In other words, he concludes that loaf volume depends on the amount of available sugar in the later stages of fermentation. Alway and Hartzell,[4] in 1909, however, performed experiments which led them to say, in contrast to Wood's findings, "there is clearly no direct connection shown between the size of the loaf and the volume of gas evolved. The thirteen flours which gave the largest loaves evolved on the average somewhat less gas than the other thirteen flours." Shutt[5] states that from his experimental evidence he was unable to find any relation between size of loaf and sugar content.

ENZYMES—Comparatively less study has been made of the enzymes of flour and their relation to strength. Perhaps the most prominent work in this field is that which was done simultaneously but independently by Baker and Hulton,[6] and by Ford and Guthrie,[7] in

[1] "Crude Gluten," *J. Am. Chem. Soc.*, **28** (1906), 8–25.

[2] "Properties of Wheat Proteins," *Ibid.*, **28** (1906), 1657–1667.

[3] "The Chemistry of Strength of Wheat Flour," *Jour. Agr. Sci.*, **2** (1907), 139–161 and 267–277.

[4] Neb. Exp. Sta., 23rd Annual Report, **1909**.

[5] "Flour—the Relationship of Composition to Bread Making Value," *Canadian Miller and Cerealist*, **5** (1913), 176–178.

[6] "Conditions Affecting the Strength of Wheaten Flour," *Jour. Soc. Chem. Ind.*, **27** (1908), 368–376.

[7] "The Amylolytic and Proteolytic Ferments of Wheaten Flour and Relation to Baking Value," *Jour. Soc. Chem. Ind.*, **27** (1908), 389–393.

1908. They point out that both proteoclastic and amyloclastic enzymes are present in flour and in many instances may exert a profound influence on its bread-making qualities. Baker and Hulton state that "it is obvious that the strength of a flour must be closely connected with the gluten, although no doubt the presence of enzymes, soluble carbohydrates, and mineral constituents all play a part." Koch,[1] in 1914, found no difference in the quantity of diastase in strong and weak flours, after extracting them with water at 0° according to the method of Thatcher and Koch.[2]

CONCENTRATION OF HYDROGEN IONS—H. Jessen-Hansen,[3] in 1911, finds a close relationship between the concentration in hydrogen ions and baking strength, and asserts that there is an optimum hydrogen-ion concentration for flour, the poorer flours having lower concentrations. He attributes the beneficial effects of neutral salts and "flour improvers" on flour to the fact that they raise the hydrogen ion concentration.

SOLUBLE PROTEINS—There does not seem to have been a very considerable amount of work done regarding the rôle of the soluble proteins as a factor in baking strength. Snyder,[4] in 1897, says "When any of the wheat proteids except gliadin or glutenin are extracted the expanding and bread-making qualities of the flour are not affected." The conclusions of Bremer,[5] in 1907, are also to the effect that the soluble proteins have little bearing on flour strength. Rousseaux and Sirot,[6] in 1913, consider the ratio of total nitrogen to soluble nitrogen as a valuable index to baking value and have determined an ideal ratio for flours according to their method, as well as the limits between which strong flours must fall in this respect.

GENERAL CONSIDERATIONS—Numerous other results of careful and valuable research might be cited, but the above serve to indicate the confusion existing in

[1] "The Diastase and Invertase Content of Wheat Flour and Their Relation to Baking Strength." Thesis for Master's Degree, University of Minnesota, June, 1914.

[2] "The Quantitative Extraction of Diastases from Plant Tissues," *J. Am. Chem. Soc.*, **36** (1914), 759–770.

[3] "Studies on Wheat Flour. Influence of H-ion Concentration on Baking Value of Flour," *Compt. rend.*, **10** (1911), 170–206.

[4] *Minn. Exp. Sta. Bull.*, **54** (1897).

[5] "Hat der gehalt des Weizemehles an Wasserlöslichen Stickstoff einer Einfluss auf seiner Backwert," *Ztschr. Unter. Nahr. Genuss.*, **13** (1907), 69–74

[6] "Les matières azoteés solubles comme facteur d'appreciation des farines," *Compt. rend. Acad. Sci.*, **156** (1913), 723–725.

the present state of our knowledge regarding the factors involved in flour strength, and is intended to serve this purpose rather than constitute anything like a complete summary of all the work which has been done in this field. Numerous summaries of this sort have been published in text-books and articles dealing with methods of milling and baking technology, such as that of the Jagos,[1] and a repetition of them here would serve no useful purpose. It is believed, moreover, that the above discussion indicates nearly all of the view-points from which the problem of the chemistry of flour strength has been attacked. The situation is very well expressed by Bailey[2] when he says: "Perhaps one of the reasons that a greater degree of success has not attended these endeavors is the fact that it has been attempted to discover one constituent (or group of constituents) which is the sole determining factor. It does not seem reasonable to believe that in so complex a substance as wheat flour the percentage of one constituent can be regarded as solely indicative of baking quality. Rather must we study these various compounds in their relation to one another, in an effort to arrive at their single and combined effects."

PURPOSE OF THIS INVESTIGATION

In a series of investigations of the various factors which may influence the strength of wheat flour, now in progress in the Division of Agricultural Chemistry of the University of Minnesota, it was proposed to study the chemical constitution of the various proteins in flour with a view toward ascertaining more definitely than has yet been done, whether or not the proteins of a strong flour may differ in their chemical constitution from those of a weak flour, since the physical properties of their glutens are found to differ so markedly.

Wood,[3] in 1907, following Osborne and Harris' modification of Hausmann's method, subjected samples of gliadin and crude gluten (composed chiefly of gliadin and glutenin) of flours of different strength, to hydrolysis for 8 hours with strong hydrochloric acid. He then steam-distilled the products of hydrolysis with magnesia and determined the percentage of nitrogen given off as ammonia. Finding a close agreement in the different samples he concluded that gliadin and glutenin of

[1] *Loc. cit.*

[2] "Relation of the Composition of Flour to Baking Quality," *Canadian Miller and Cerealist*, 5 (1913), 208–209.

[3] *Loc. cit.*

all wheat flours are of the same chemical composition. since the work of Wood,[1] a more detailed method of protein analysis, which gives further insight into the constitution of the protein molecule and is capable of yielding quantitative results, has been presented by Van Slyke,[2] who has incidentally shown that the hydrolysis of gliadin with strong hydrochloric acid is not complete at the end of 8 hours. It was therefore decided to make further study of the chemical constitution of flour proteins in the light of better methods of analysis now available.

METHODS OF STUDYING CHEMICAL COMPOSITION OF PROTEINS

It has been shown repeatedly that for practical considerations all of the nitrogen of flours of the higher milling grades may be regarded as in the proteins. The chemical structure of the proteins has been clearly demonstrated by Fischer[3] and a host of other workers since, so that it needs no elaborate discussion here. Briefly stated, the facts appear to be that the protein molecule is made up of a number of amino acids, there being some 18 or 20 of these which occur in natural proteins. These are probably linked together by anhydride combinations between the amino group of one amino acid and the carboxyl group of another. This is indicated by the nature of the products formed (amino acids) when the protein is subjected to hydrolysis. Moreover, it appears that the characteristic chemical and physical nature of individual proteins depends largely on the nature and number of the various amino acids of which they are composed. In a comparison of the chemical constitution of proteins, then, it is necessary to split the molecule by hydrolysis into its "bausteine" (characteristic units) and determine the relative proportions of these which are formed in each case. There is no known method of ascertaining the exact manner in which these units are grouped together in the various proteins, since even the sensitive anaphylaxis reaction is not specific in the case of many vegetable proteins, as has been demonstrated by Wells and Osborne,[4] who found that animals

[1] *Loc. cit.*

[2] "The Analysis of Proteins by the Determination of the Chemical Groups Characteristic of the Different Amino Acids," *J. Biol. Chem.*, **10** (1911), 15–55.

[3] "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Berlin, **1899–1906**.

[4] "Is the Specificity of the Anaphylaxis Reaction Dependent on the Chemical Constitution of the Proteins or on Their Biological Relations? The Biological Reactions of the Vegetable Proteins. II," *Jour. Infect. Dis.*, **12** (1913), 341–358.

sensitized with gliadin of either wheat or rye will react with hordein of barley, a protein known to have a different chemical constitution, and that gliadin and glutenin, known to be different as regards the relative proportions of the various amino acids in their molecule, react anaphylactically with each other.

METHOD USED IN DETERMINING PRODUCTS OF PROTEIN HYDROLYSIS

Van Slyke's method gives the most detailed insight into the protein molecule of any known method which, at the same time, indicates quantitatively the distribution of its component units. Accordingly, the Van Slyke method, in some cases slightly modified, was used in this investigation. The method, which is an extension of the principle of the Hausmann method, consists of a division of the protein molecule into various groups, or units, after prolonged hydrolysis with hydrochloric acid, and the determination of the percentage of nitrogen in each individual group, thus ascertaining the distribution of the total nitrogen in the protein. Briefly, the groups determined are: (1) ammonia or amide nitrogen, which is considered to be derived from $—CONH_2$ or —CONHOC— groups linked to the carboxyl groups of the dicarboxylic acids in the protein molecule (glutamic and aspartic acids); (2) humin nitrogen, from the dark-colored pigment and slight amount of insoluble matter always formed in the hydrolytic products of acid hydrolysis of proteins; (3) the amino nitrogen of the mono-amino acids, which corresponds to all of the mono-amino acids excepting proline and oxy-proline; (4) the non-amino nitrogen of the mono-amino acids, which corresponds to the proline and oxy-proline; and (5) to (8) the nitrogen corresponding to each of the individual di-amino acids, *i. e.*, arginine, lysine, histidine, and cystine, respectively. Thus, eight units of the protein molecule may be estimated quantitatively, the determination of histidine nitrogen and lysine nitrogen being subject to a larger experimental error than the other units, which may be determined with the exactness required by ordinary quantitative procedure.

FLOURS USED IN THE INVESTIGATION

Eight flours of the higher grades (as separated in the process of milling) from various sources and of varying baking qualities were selected for the preliminary work. Their sources and relative baking values, as measured by loaf volume, are indicated in Table I.

TABLE I—DISTRIBUTION OF NITROGEN IN THE PRODUCTS OF HYDROLYSIS OF ENTIRE FLOURS

Sample No.	Flour	Source	Baking Strength Loaf volume	Total N Per cent	Per Cent of Total Nitrogen Ammonia N	Humin N	Basic N	Monoamino acid N
B401	Pillsbury Patent	Northern Sprin g Wheat	Good	2.035	20.81	5.32	8.10	65.77
B438	Patent Biscuit Flour	Soft Missouri Wheat	Poor	1.67	18.85	5.62	9.25	65.65
B439	Patent Flour	Nebraska Turkey Wheat	Good	1.928	21.01	4.92	8.56	65.51
B440	Patent Flour	Hard Wheat, Prosser, Washington	Medium	2.170	21.47	5.00	7.88	65.65
B441	High Gluten, Low Strength Flour	North Dakota Wheat	Poor	2.13	21.03	5.16	8.74	65.07
B444	Very High Gluten Flour	Kansas Experiment Station	Medium	2.55	23.00	5.42	7.08	64.50
B445	"Fortyfold" Soft White Wheat	Troy, Idaho	Very Poor	1.260	18.21	7.51	9.00	65.28
B452	Patent Flour	Ritzville, Washington	Poor	1.917	19.87	5.63	8.03	66.47

THE PRODUCTS OF PROTEIN HYDROLYSIS FROM ENTIRE FLOUR

Osborne[1] and his associates have shown that there are five proteins present in flour, *viz.*, gliadin, glutenin, albumin, globulin and proteose, the latter being of little significance. The first two named compose the gluten, already referred to, while the others are soluble in dilute salt solutions and are, for the most part, removed in the familiar process of "washing out" the gluten.

Since the proteins are, for all practical considerations, the only nitrogen compounds in the higher grade flours, it was decided to submit first, in all cases, a sample of the entire flour to prolonged hydrolysis with strong hydrochloric acid, and determine the distribution of nitrogen in the various units. Should the results vary in different flours, it would be necessary to obtain the different proteins and ascertain their composition in a similar manner. If they should show the same chemical constitution then they must be present in the flour in varying amounts to account for the difference when analyzed collectively, as is done in the hydrolysis of the entire flour. That the latter is true has, of course, been concluded by numerous investigators who have extracted flour proteins with specific solvents and have found their amounts to vary widely in different flours. The solvents most frequently used are: (1) alcohol—varying from 50 to 80 per cent, and (2) neutral salt solutions of different concentrations. The former was at first thought to extract only gliadin while the latter was considered to remove only albumin, globulin and proteose. Owing to the fact that solutions of varying strengths and different methods of extraction have been employed by different investigators, however, their results often disagree widely, and in many cases even fail to support the same general conclusions. Furthermore, it has been found that the solvents mentioned above are not as specific as was formerly supposed, and that alcohol extracts not only gliadin but also considerable of the "soluble proteins," the material so extracted depending on the strength of the alcohol, while salt solutions extract some gliadin as well as albumin and globulin, according to the concentration of the solution. Other physico-chemical factors undoubtedly enter as well. Olson[2] states that

[1] "The Vegetable Proteins" (1912), Plimmer's, Monograph, London, New York, etc.

[2] "Quantitative Estimation of Salt-Soluble Proteins in Wheat Flour." *J. Ind. Eng. Chem.*, **6** (1914), 212.

"the amount of gliadin extracted by 1 per cent sodium chloride solution approximately amounts to about 29 per cent of the total proteids," and "the nitrogen bodies soluble in salt solution are partly or wholly soluble in diluted alcohols varying with the concentration of sodium chloride used." That a study of the products of hydrolysis of the flour proteins both collectively and individually can furnish an indication of the proportions of these proteins in the flour, providing there is no difference in the chemical constitution of the same proteins in different flours, is evident from the following considerations: the percentage of ammonia nitrogen yielded on the hydrolysis of the individual proteins of wheat flour varies as follows, according to Osborne, gliadin 24.5, glutenin 18.8, leucosin (albumin of flour) 6.8, and globulin, 7.7. Since the figures for the ammonia nitrogen show wider variation than do those of any other units, and since also the estimation of this unit is probably accompanied by less error than that of any of the others, it may be supposed that its estimation in the proteins taken collectively and individually will indicate closely the relative amounts of the various proteins present, providing, as mentioned before, the same proteins of different flours do not vary in their chemical constitution.

In determining the distribution of nitrogen in the entire flour, 10-gram samples were hydrolyzed for 48 hours, and the "Hausmann" units determined. In the case of the entire flour the presence of a large amount of starch occasions a voluminous precipitate of "humin" material which made it impractical to attempt a determination of all the units of the Van Slyke method, since a large enough sample could not be used to insure the estimation of the smaller units with sufficient accuracy that the figures would be of much significance. The instructions of Van Slyke regarding the conditions for precipitating and washing the bases, however, were carefully followed.

In determining the total nitrogen in the hydrolyzed mixture, the presence of large amounts of "humin" substances resulting from the carbohydrates, and small amounts of fat, necessitated the slight modification of Van Slyke's method suggested by Gortner.[1] The above mentioned substances make it impossible to

[1] "Studies on the Chemistry of Embryonic Growth. I. Certain Changes in the Nitrogen Ratios of Developing Trout Eggs," *J. Am. Chem. Soc.*, **35** (1913), 632–645.

obtain an aliquot until after they have been removed in the processes of determining the ammonia and humin nitrogen. Consequently, the hydrolyzed mixture is evaporated *in vacuo* to remove most of the hydrochloric acid. The ammonia is distilled off as in the Van Slyke process (without removing the material from the distillation flask from which the acid was evaporated off), collected in standard acid, and estimated by titration; the humin filtered, washed, and submitted to Kjeldahl analysis for nitrogen, and total nitrogen determined in aliquot portions of the filtrate from the humin. This, added to the ammonia nitrogen and the humin nitrogen, gives the total nitrogen in the hydrolyzed sample. No correction was made in any of the analyses for the solubilities of the bases in the solutions from which they were precipitated, since the same conditions were observed in all cases and the results are strictly comparable.

The results given in Table I were obtained from the analyses of the eight samples of flour by the above-described process. The different flours vary significantly with respect to the ammonia nitrogen yielded on hydrolysis. The basic nitrogen or nitrogen of the diamino acids also shows a slight variation, this being inversely as the variation in ammonia nitrogen. The variations shown in the table are much greater than could possibly be due to experimental error and were confirmed by repeated determinations. Hence, there can be no doubt that these variations show actual characteristic differences in the nitrogen distribution in the different samples.

THE DISTRIBUTION OF NITROGEN IN GLIADIN, GLUTENIN, AND SOLUBLE PROTEINS

Hydrolysis of the entire flour having shown characteristic differences in the composition of the entire protein material contained in them, it appeared to be necessary to establish as definitely as possible whether or not the chemical constitution of the various individual proteins is the same in different flours. For this purpose two flours which differed widely in their origin, total nitrogen content, and baking strength were selected. Flour B401 is a typical Minnesota patent flour, milled from northern spring wheat, of fairly high nitrogen content and of good baking strength, while B438 is a patent biscuit flour, made from a softer Missouri wheat, low in total nitrogen and of poor baking strength. Gliadin was extracted from the

gluten of the flours with alcohol and carefully purified by pouring the concentrated syrup from the clear alcoholic extract alternately into large volumes of water and strong alcohol and finally digesting with absolute alcohol and ether, according to the method of Osborne. Glutenin was also prepared according to Osborne's method which consists, briefly, of dissolving the residue left after the alcohol extraction of the crude gluten in a dilute solution of potassium hydroxide, neutralizing with hydrochloric acid to precipitate the glutenin, decanting the liquid and further extracting the precipitate repeatedly with alcohol to remove the remaining gliadin; finally digesting with absolute alcohol and ether. The preparations of glutenin in this work were not pure, being contaminated by small quantities of carbohydrates, owing to lack of facilities for obtaining clear extracts and filtrates at the time, but it is believed that all other nitrogen-containing bodies were removed, and that the preparations served the purpose of the investigation, namely, to ascertain whether there was any appreciable difference in the chemical constitution of the pure proteins. Considerable quantities of each of the two flours were then extracted with 1 per cent salt solution, the extracts were filtered as clear as possible and concentrated *in vacuo*. These extracts and weighed quantities of the gliadin and glutenin were then hydrolyzed for 48 hours with strong HCl. The gliadin and glutenin were analyzed according to the Van Slyke method, while only ammonia nitrogen was determined in the case of the soluble proteins. From the results shown in Table II (1, 2, and 3) it is readily seen that, after making allowance for the limits of experimental error of the method, there is no apparent difference in the chemical constitution of the proteins of typical strong and weak flours of the same market grade.

THE DISTRIBUTION OF NITROGEN IN CRUDE GLUTEN

More complete evidence that the gluten-forming proteins are of the same chemical constitution in different flours was obtained by analyzing thoroughly washed crude glutens of three flours of widely differing characteristics. The same two flours as in the immediately preceding experiments were used, and in addition, B444, a Kansas flour of exceedingly high nitrogen and gluten content, but of low baking strength, as shown in Table I. The results, obtained from the complete Van Slyke process as applied to the crude glutens from these three flours, appear in Table II (4) and indicate

that not only are the gluten-forming proteins in flours of widely differing baking qualities of the same chemical constitution, but the ratio of gliadin to glutenin is probably the same, or very nearly so, in flours of the same market grade but very different baking strengths. With respect to this latter point, it may be said that since, as is shown above, gliadin yields 26 per cent of its nitrogen as ammonia nitrogen after hydrolysis, while glutenin yields only 16 per cent of its nitrogen in this fraction, the determination of ammonia nitrogen of the hydrolyzed glutens will certainly indicate any significant variation in the ratio of gliadin to glutenin in different flours, although the limits of experimental error are not narrow enough to indicate very small variations in this ratio. The data in Table II (4) indicate very clearly, therefore, that there is no significant variation in the gliadin-glutenin ratio in flours of such widely varying baking strength as those used in this investigation.

THE SIGNIFICANCE OF THE SOLUBLE PROTEINS IN AFFECTING THE NITROGEN DISTRIBUTION IN FLOUR

It is evident that the differences in the percentages of ammonia nitrogen and basic nitrogen yielded on the hydrolysis of the several entire flours, as shown in Table I, cannot be accounted for as being due to differences in chemical composition of the individual proteins since it has been clearly shown that these have the same chemical constitution.

It was thought at first that the varying percentages of starch in the different flours might cause differences in the percentage of ammonia nitrogen, since Mann,[1] in 1906, states "if in addition to the carbohydrate, ammonia or other nitrogenous substances are in solution, then the humins combine with the ammonia and thereby become nitrogenous." In order to ascertain whether varying proportions of starch would influence the results obtained by the Van Slyke method as used in these investigations, a sample of the flour B401, to which had previously been added 20 per cent of its weight of wheat starch, was hydrolyzed and the distribution of nitrogen in the products of hydrolysis determined. There was no significant change in the percentage of ammonia nitrogen when compared with the sample to which no starch was added, although there was a very noticeable increase in humin N and a corresponding decrease in basic N, as shown in Table III.

[1] "Chemistry of the Proteids," New York, 1906.

BIOGRAPHICAL

Morris J. Blish was born April 21, 1889, at Lincoln, Nebraska. He attended the public schools there, through the seventh grade. He then moved to Omaha, Nebraska, where he graduated from the Omaha High School in 1906. After working in one of the Omaha banks for a year, he entered the University of Nebraska in the fall of 1907, specializing in chemistry and graduated with the B.Sc. degree in February, 1912. Enrolling in the graduate school of the University of Nebraska, he received the A.M. degree in agricultural chemistry, June, 1913, thesis work having been on soil chemistry under the direction of Dr. F. J. Alway. He received an appointment as research assistant in agricultural chemistry at the University of Minnesota, and enrolled in the graduate college of that university in September, 1913, working under the direction of Prof. R. W. Thatcher. He received the Ph.D. degree in June, 1915, thesis work having been on the chemical constitution of the proteins of wheat flour, in relation to "baking strength."

PUBLICATIONS

1. On the Distribution and Composition of the Humus of the Loess Soils of the Transition Region.

2. On the Origin of the Humin Formed by the Acid Hydrolysis of Proteins. (In collaboration with Dr. R. A. Gortner.)

3. Concerning the Identity of the Proteins Extracted from Wheat Flour by the Usual Solvents. (In collaboration with C. H. Bailey.)