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Review of Proteins of Wheat Flour

The elastic, cohesive properties contributed to doughs by the proteins of wheat flour are basically responsible for the characteristic porous structure of leavened baked products. The chemical and physical properties of this very complex group of components are ill defined. The multicomponent albumin and globulin proteins vary significantly in amount among different flours and appear of much potential importance as enzymic or structural modifiers of the complete complex of proteins in doughs. The lipides, and possibly the soluble proteins, present in gluten as ordinarily obtained from doughs are integral parts of the complex and necessary for normal behavior of this most important fraction of flour.

THE PROTEINS OF WHEAT FLOUR are responsible in large part for the dominant position held by wheat among cereal food grains used by western peoples for many centuries. A principal reason for such popularity is the exclusive ability of wheat proteins to provide a structural framework for the familiar spongy, cellular texture of bread and a wide variety of other baked products. The very nature of these proteins that is responsible for their usefulness, however, also has caused knowledge of their fundamental character to lag far behind their highly developed utilization based on empirical findings.

Flour proteins are usually considered to belong, in a general way, to a soluble or an insoluble fraction, although this classification is known to be inexact. The so-called insoluble fraction, known as crude gluten, contains a complex mixture of proteins, fats, and carbohydrates and is ordinarily obtained by carefully washing doughs. The soluble fraction, obtained directly from flour by extraction with water or a salt solution, is also complex. The extent to which protein components from each fraction can intermingle with those of the other has only recently become appreciated, and both fractions contain extraneous materials that are difficult to remove without altering the nature of the proteins themselves. Such factors as these, among others, have seriously hindered definitive studies on all flour proteins.

General knowledge of flour proteins has been ably summarized in a number of reviews (1, 4, 27, 29, 40), the most recent of which was presented before the AMERICAN CHEMICAL SOCIETY in 1954 by Betty Sullivan in her Garvan Medal address (40). The present paper is consequently confined to more specific aspects that supplement and enlarge upon the prior discussions, particularly with respect to the soluble proteins.

Results obtained in the authors'

laboratory in recent years have furnished some new criteria of identity for soluble proteins that are useful in resolving some of the confusion regarding flour proteins that exists in the literature. For this reason, the usual order of presentation is reversed, and soluble proteins are discussed before gluten.

Albumin and Globulin Proteins

Classically, the soluble protein fraction of flour was regarded as a simple mixture consisting of, for instance, an albumin, and a poorly defined material called "proteose" by Osborne (29). Modern methods, however, permit demonstration of several components in both the albumin and globulin fractions (8, 19, 37). Ultracentrifugation showed the presence of three different globulins in purified preparations isolated from flour (8, 19), and electrophoresis on filter paper showed 11 discernible components in the albumin fraction (30). The individual components have not yet been separated from either fraction on a practical scale. The usual methods of fractional precipitation with salts or organic solvents, etc., have failed to give satisfactory results, but newer physical methods such as continuous electrophoresis on filter paper hold much promise for the future.

Determination and Occurrence

However sensitive modern physical methods are for qualitative detection of minor components, they are usually cumbersome, expensive, or otherwise unsuited for routine quantitative estimation of either total soluble proteins or the amount of any of the individual components present in a flour. The variation in amounts of individual components visible in sedimentation diagrams or paper electropherograms may very well be related to important differences in baking properties that exist among flours, so that an

accurate, convenient method of analysis is highly desired. At present only total amounts of albumins and globulins in flour can be estimated with any confidence (except, perhaps, β -amylase, as discussed below).

Little is yet known regarding the amino acid composition of soluble proteins, but average amounts of certain amino acids of particular interest are included in the following table, where values are expressed as percentage of the dry preparations.

Factor	Albumins	Globulins
Total N	17.1	18.6
Amide N	1.7	1.4
Tryptophan	3.23	0.99
Arginine	8.7	17.0

The high total nitrogen and arginine contents of the globulin fractions are similar to those of other seed globulins such as edestin and amandin. The arginine content varied, however, among preparations of both albumins and globulins known to have different proportions of individual components, as determined in the ultracentrifuge or by electrophoresis on paper. On the other hand, amide nitrogen and tryptophan contents were relatively constant among the preparations. The high tryptophan content of albumins distinguishes them from other flour proteins, and the low amide nitrogen contents of both fractions serve to distinguish them from gliadin, the only other protein apt to occur to a significant extent in water or dilute salt extracts of flour. These characteristic differences in amino acid composition constitute the basis for a method of quantitatively estimating total albumins and total globulins in flour (35).

A large number of flours of widely varying type and baking quality were analyzed for albumin and globulin.

The total soluble protein contents ranged from about 13 to 22% of total flour protein, and there was usually slightly more albumin than globulin present. Values typical of those obtained are as follows:

Flour Type	Per Cent of Flour N			Gluten (by Dif- ference)
	NPN	Albu- min	Globu- lin	
Soft white	2.5	10.9	7.6	79.0
Soft red	1.6	10.7	7.8	79.9
Hard red winter	2.0	9.1	5.7	83.2
Hard red spring	1.8	7.8	5.8	84.6
Durum	2.2	6.8	7.2	83.3

Both albumin and globulin contents, as well as the ratio of albumins to globulins, varied significantly among the more than 40 flours so far examined in this manner. Curiously, only the albumin-globulin ratios were correlated significantly with a measure of baking quality applied to the protein systems of the flours (36).

The above analytical method for albumins and globulins was also used to clarify some of the results reported by earlier investigators regarding soluble protein contents of flours. Gortner, Hoffman, and Sinclair (12), for instance, found that the kind and amount of salt used affected the amount of nitrogen that could be extracted from flours. Pence, Weinstein, and Mecham (35), however, showed that solubilities of albumins, globulins, and gliadin are all influenced by salts present in extracting solutions, but because gliadin is present in such larger amounts, effects associated with it are apt to obscure other changes. For example, the amounts of protein extracted by 0.5M solutions of the following halide salts varied much more for gliadin than for the other two types of protein:

Salt	Percentage of Flour Protein Extracted as			
	Total pro- tein	Albu- min	Globu- lin	Glia- din
NaF	12.0	6.0	4.7	1.3
NaCl	21.4	7.6	7.6	6.2
NaBr	32.7	9.6	6.5	16.7

Distilled water or very dilute salt solutions extract albumins more or less completely, but they extract practically no globulin and variable amounts of gliadin, depending somewhat on the particular flour used. Fortunately, gliadin is easily removed from such extracts by salting out with low concentrations of ammonium sulfate, and albumins and globulins can be separated satisfactorily by dialysis. Removal of contaminating hemicelluloses during purification of extracts, however, so far is accomplished only with loss of significant amounts of protein material.

Although methods for separation or quantitative analysis of individual albumin or globulin components have yet to be developed, visual examination of paper electropherograms shows clearly that different albumin preparations may contain varying amounts of some of the components, depending on the flour used and the manner of preparing the fractions. Of particular interest is the finding that albumins from durum flours show a characteristically different distribution of components (37). Durum flours appear to have a greater proportion of α -albumins and lower proportions of β -albumins than flours from common or club wheats. The differences observed were independent of growth location and crop year.

Possible Functional Properties

The function of soluble flour proteins in the overall behavior of doughs eludes satisfactory explanation for the most part, although they are recognized to be related to bread baking properties of flours, as shown by the work of Finney (17) and Pence and coworkers (32, 35). Probably many of the soluble proteins are enzymes. For instance, the proteinase of flours described by Balls and Hale (2, 13) probably occurs in the globulin fraction, to judge from the procedure described for isolating it. Recent preliminary findings at this laboratory also indicate that β -amylase is probably the α -2 (30) component of the albumin fraction. If this is confirmed by future work, α -2-albumin would be the first of the albumin components that could be specifically determined in flours, as reliable methods for β -amylase are in common use (17).

Isoelectric points and pH-mobility curves determined for albumin components (30) clearly show that the various components may have substantially different amino acid compositions, but very little information of this sort is yet available. The cystine content of a few albumin preparations has been found to be as high as 6% of the preparation, but usually it is lower. Like arginine, cystine appears to vary in amount, depending upon the proportions of different components in a particular preparation. Globulins are low in cystine, running less than 0.4% in some instances. Both albumins and globulins show sulfhydryl contents of the order of 0.1% (expressed as cysteine), as determined by the amperometric titration procedure of Koltzoff, Stricks, and Morren (18). However, globulin preparations generally have a higher sulfhydryl content than albumin preparations.

An interesting fractionating effect of urea and sodium salicylate on the cystine content of albumin preparations has been observed in this laboratory. In 15% sodium salicylate the average molecular weight of a preparation appeared to be about half of that in 0.1M

sodium chloride. When the salicylate was replaced by sodium chloride, the residual protein regained its original molecular weight. However, during the interchange of the salts, as much as 30 to 40% of the protein became insoluble. The cystine content of the fraction remaining soluble was found to have become enriched, while that of the precipitate was very low. Attempts to confirm a selective action on particular components by electrophoresis on paper have so far been inconclusive.

This review of what is known of the soluble proteins emphasizes how much we really should know of this class of flour constituents. They appear to be a group of materials with much potential importance with respect to flour utilization and technology, because some of them are enzymes and others may substantially modify gluten behavior, even if they may not be considered structural materials in the protein framework of bread. It is conceivable that oxidizing agents influence dough behavior by way of the soluble proteins. Albumins are known to be important to flour quality in that they are needed in reconstituted doughs, but nothing is yet known of the possible function of globulins.

Gluten

Far more attention has been paid to the insoluble, or gluten, fraction of flour proteins over the years than to albumins and globulins. Two reasons for this are obvious. First, the unique cohesive and elastic properties of gluten permit ready separation of the bulk of flour protein from nearly all nonnitrogenous constituents, thereby overcoming a major problem in the study of plant proteins. Secondly, its physical properties suggest immediately that gluten provides the basic framework for the desirable structure of baked products and thus should merit primary attention. Despite this extensive investigation of gluten proteins, no satisfactory explanation has emerged for their physical behavior or for variations in physical properties that appear responsible for wide variations in baking characteristics of flours from different wheat varieties and sources.

The rubbery mass of crude gluten is usually obtained from flour by kneading a dough in water to wash away other constituents, and it consistently contains about two thirds by weight of water and one third dry matter. The dry solids contain 75 to 85% protein and 5 to 10% lipide with occluded starch or starch fragments making up most of the remainder. The lipide appears to be combined with protein in a manner typical of lipoproteins, in that alcohol or similar solvents are necessary to extract them. In fact, the term "lipoglutenin" was proposed by Olcott and Mecham

(28) for that fraction of protein binding most of the lipide.

Formation and Separation

For many years chemists regarded gluten as a mixture of approximately equal parts of gliadin and glutenin. These terms are now known to be improper, because they do not designate definite chemical substances, but the terms are almost universally retained for the sake of convenience. Gliadin refers to that portion of gluten soluble in 60% aqueous alcohol, and glutenin to the remainder, which is insoluble in neutral solvents but soluble in acidic or alkaline solvents.

A great many studies conducted on gluten as ordinarily obtained from flour have failed to recognize the possibility of incomplete removal of soluble proteins during the washing of doughs, although McCalla and Rose (22) called attention to small amounts of soluble proteins in solutions of crude gluten 20 years ago. Analysis of glutes and their wash waters performed in the authors' laboratory confirms these findings. The results below show that approximately half of the total albumins and nearly all of the globulins of a flour may be retained by crude gluten when washed from doughs using a 0.1% phosphate buffer at pH 6.8.

	Flour 1, Hand- Washed	Flour 2, Blender- Washed
Analytical gluten protein content of flour, %	(85)	(84)
Flour protein recovered as crude gluten, %	93	90
Protein content of crude gluten obtained, %	82	98
Albumin retained in crude gluten, %	49	60
Globulin retained in crude gluten, %	89	51

Even vigorous washing by means of a mechanical blender to give a gluten practically free of starch fails to remove more than about half of the soluble proteins from the gluten. At the same time as much as 2% of the total flour protein may be removed in the form of soluble gliadin. An actual binding of soluble protein, particularly albumins, to gluten during dough formation is suggested by these results.

Nearly complete separation of soluble proteins from gluten can be accomplished by using wash solutions up to 0.5M in salt concentration or by extracting a flour with a salt solution before making it into a dough. The glutes obtained, however, are likely to be abnormal. With the preliminary extraction procedures the authors found doughs were

difficult to wash, and in both cases the glutes were short and granular. They failed to give normal loaf volumes when reconstituted into doughs and baked, even though precautions were taken to ensure proper gas production and to keep the level of salt in the doughs near normal.

Failure to remove soluble proteins completely during preparation of glutes might be of small consequence if there were not several indications of important modifying effects of soluble constituents and other materials on the properties of gluten. An example concerns the physical behavior of doughs, as compared to glutes, during a rest period. Dempster, Hlynka, and Anderson (9, 10), among others, have shown how the internal stress of a dough diminishes during rest. Udy (47), on the other hand, showed that purified glutes become more resistant to stretching after resting, as contrasted to doughs which mellow and soften as a result of the relaxation of their internal stresses during resting. Another example concerns the effect of soluble flour fractions on baking properties of reconstituted doughs reported by Finney (11) and by Pence, Elder, and Mecham (32). Baking properties of crude glutes obtained from flours of widely varying properties were rather similar when soluble fractions were omitted from reconstituted doughs. Original differences in quality were restored when the soluble materials were added back to the gluten and starch doughs.

These examples emphasize the necessity of a clear distinction between purified gluten to be used for characterization studies on the proteins themselves and crude gluten for study of the complexed system more nearly representing the condition existing in doughs, if not the flour itself.

Many cereal chemists believe that crude gluten may not represent the condition or composition of proteins in the original flour. The most recent advocate of this view is Hess (14-16), who has presented convincing evidence that gluten proteins occur in flour in a much different form than in crude gluten. He has separated two proteins from flour by differential sedimentation in nonaqueous liquids. "Wedge" protein occurs between starch granules in the endosperm cells of wheat. The other occurs as a network of fibrils on the surface of starch granules and is covered with a layer of lipide; this fraction he calls "adhering" protein. The wedge protein is readily separated from starch, but the adhering protein clings tightly to the granule during the isolation procedures. Differences in x-ray diffraction patterns before and after swelling in water clearly distinguish these proteins from gluten as normally obtained from doughs. A structural chemical alteration of flour

protein appears to be caused by mechanical influences during the conversion of the proteins to gluten. Wedge protein is slightly more acidic than adhering protein, having an isoelectric point near pH 6 and no free amino groups that react with formalin. Adhering protein had an isoelectric point near pH 7, as judged by turbidity measurements.

Chemical Characterization

Problems in the physicochemical characterization of gluten have some counterparts in certain aspects of its chemical characterization. The amino acid composition of crude gluten is known, and the usual amino acids account reasonably satisfactorily for the nitrogen present. However, the amino acid composition of crude glutes from a wide variety of flours was surprisingly uniform and failed to reflect differences in dough characteristics or baking quality of the flours (33). This may have been due to the uniformity of the standardized procedure used to prepare the glutes; more variation probably would have been observed had the total flour proteins been analyzed so as to include all the varying amounts of soluble proteins contained by the flours (36). With whole wheats, for instance, McElroy and coworkers (23) and Price (38) found significant variations for several amino acids that correlated with total protein. This was interpreted by Price to indicate varying ratios of the different proteins. Miller and others (26), likewise, found a significant variation in cystine and methionine among wheats grown in different environments and in different crop years.

Cereal chemists probably have had the greatest interest in the amino acids cystine and cysteine in flour proteins because of the importance of cystine disulfide bonds to the desirable physical properties of gluten. Reagents capable of breaking disulfide bonds cause a rapid loss of elasticity and an increased fluidity and stickiness of doughs to which such reagents are added. Probably only a small fraction of the total disulfide bonds are of major importance to the structural integrity of gluten, because fragmentation of the molecules by reducing agents occurs to but a slight extent in the gliadin fraction of gluten (34).

Other evidences of a relation between the cystine and cysteine content of wheats or flours and their baking properties are reported by several workers. For example, Miller and others (26) found a correlation between cystine contents of hard red winter wheats and mixing times of flours derived from the wheats. Wöstmann (45) reported a correlation between cystine content of flours and resistance to extension in doughs as measured by the area under extensograms.

The sulfhydryl group of cysteine is also of considerable interest to cereal chemists because of its relation to the improving action of certain oxidizing agents on dough properties. Very small quantities of an oxidant, such as potassium bromate, are used commercially to make doughs more elastic and cohesive, and less extensible, an effect opposite to that caused by reducing agents. The kinship of sulfhydryl groups to the disulfide bonds of cystine and the reactivity of sulfhydryl groups to mild oxidation make them the most likely group in flour proteins to be associated with rheological changes observed upon oxidation. A completely satisfactory explanation of how these effects are brought about has yet to be established. Moran, Pace, and McDermott (27) found cysteic acid and oxides of methionine to be principal products of the reaction of chlorine dioxide on flour proteins which survived subsequent hydrolysis of the fractions required for analysis.

A principal difficulty in demonstrating the relation of sulfhydryl groups to the oxidation improvement of flours is the difficulty in showing a reasonable quantity of sulfhydryl groups in flours and gluten. The low level of reducing activity present is below the level at which common analytical methods are accurate. At present, amperometric titration with a rotating platinum electrode seems to be the method of choice (28). Measurement of possible sulfhydryl groups in gluten is especially difficult because of the problem of keeping the protein in solution during the determination. A detailed treatment of these and related problems was presented in 1954 by Sullivan (40).

Recently, Wiseblatt, Wilson, and McConnell (44) have reported studies in which some of the newer methods available for study of the structure of proteins were applied to gluten. Peptide chains were broken selectively at the amino groups of serine residues. The peptides displayed little electrophoretic heterogeneity; but this was attributed at least in part to the introduction of numerous acid groups. Most of the serine appeared at the amino ends of the peptides; and although osmotic pressure measurements indicated an average molecular weight of 20,000, terminal group estimates indicated that each molecule contained several *N*-terminal serine residues. Consequently there appeared to be strong association or chemical cross linking between peptide chains of the degraded gluten. The authors pointed out that the formation of cross links between chains during the degradation has not been ruled out. If the associations or cross links are those present in ungraded gluten, however, separation of these polypeptide fragments should expedite their identification.

Relations with Lipides

A particularly interesting and significant aspect of the chemistry of normal gluten is the importance of the nature and amount of lipide associated with it upon physical properties of the complex. The lipoprotein nature of at least part of gluten was mentioned above, and detailed discussion of the subject is given in the reviews of McCalla (27) and Coppock and coworkers (6, 7).

In flours, most of the 1 to 2% lipide present is easily extracted with ethyl or petroleum ether, but alcohol or other solvent capable of splitting lipoprotein bonds is required to extract total lipide from flour. After doughing, Olcott and Mecham (28) found 70 to 90% of the lipide unavailable to ether extraction. The lipide of crude gluten consequently is nearly all bound by the time it is washed from a dough.

McCalla and coworkers (20, 39) showed that the lipide most difficult to extract from flour (principally phospholipide?) was of greatest importance in forming gluten of normal properties, and suggested that the lipoprotein complex occurred in flour or was formed during washing of the gluten. Olcott and Mecham (28) found that both situations occurred, and that the least soluble portion of gluten (glutenin) was responsible for binding the lipide. Mecham and Weinstein (25) later found that lipide was bound by gluten to a reduced extent because of the salt present in bread doughs. The effect of salt may indicate an electrostatic type of binding between the protein and lipide.

Hess (14, 15) has recently confirmed the importance of phospholipide for normal characteristics of flour and dough. He found phospholipide ("lecithin") to be intimately associated with both wedge protein and adhering protein, but particularly with the latter. A layer of neutral lipide appears to lie between and to separate the network of protein adhering to starch granules and the outer lamellae of wedge protein, whereas phospholipide appears to be an integral part of the fibrillar structure of the protein network itself. Normal lattice expansion on x-ray diagrams for hydrated gluten separated from a dough was obtained only when the lipide was present in the flour before doughing. Flour free of phospholipide would not even form a dough.

A major difficulty in determining the true effect of bound lipide on baking properties of flour has been the required use of alcohol to remove total lipide, resulting in an apparent and complicating denaturation of some of the flour protein. Recently, Mecham and Mohammad (24) found that water-saturated *n*-butyl alcohol efficiently released the total lipide of flour without causing

extensive denaturation of the proteins. After extraction with this solvent, gluten could be washed from flour and appeared to be as elastic, although less extensible, than gluten washed from unextracted flours. Baking properties of the proteins were affected to a significant extent, but not as drastically as if ethyl alcohol had been used to extract the lipide.

Any discussion of lipoproteins in flour should include a word concerning purothionin, the crystalline protein isolated from a petroleum ether extract of flour by Balls, Hale, and Harris (3). This curious substance could be found in no other fraction of wheat or flour besides the endosperm and only in the ether-extractable fraction of the lipide, even then in very small amount. The high cystine content of this protein, almost 16%, suggests a relation to the oxidation improvement of flours, but no connection with any mechanism has been demonstrated. When freshly isolated, the crude material gives a strong nitroprusside reaction, but the crystalline material shows no evidence of free sulfhydryl groups. Another interesting feature of this protein is its basic reaction, due to a high content of arginine.

A recent series of papers, somewhat unrelated to the preceding topics, is worthy of brief comment, principally because of growing concern with public health aspects of food materials. Winteringham and associates (5, 42, 43) have studied the products formed in wheat and flour as a result of fumigation with methyl bromide. Nearly all of the reagent is taken up by the protein fraction. The end products appeared to consist almost entirely of inorganic bromide, *O*-methyl, *S*-methyl, and dimethylsulfonium compounds, and *N*-methylhistidine derivatives. More than half of the reaction products were those of histidine. However, preliminary considerations indicated no evidence that the products would be toxic or that their formation resulting from normal fumigation treatments would result in any significant reduction in amount of essential food constituents.

The foregoing discussion has reviewed briefly certain aspects of our present knowledge of the nature of flour proteins and their relationships with one another. Of necessity a great many things were omitted. The sum of our knowledge concerning these important substances is scant in the face of the huge gaps remaining, but actually the outlook has never been brighter. With the rapid advances in knowledge of proteins in general and with the powerful new methods that are becoming available, equally important advances in knowledge of flour proteins are inevitable, even though the protein systems may turn out to be even more complex than we may care to contemplate.

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Received for review October 10, 1955. Accepted March 7, 1956. Division of Agricultural and Food Chemistry and American Association of Cereal Chemists, Symposium on Cereals, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

CEREAL COMPONENTS

A Review of Carbohydrates of Wheat and Other Cereal Grains

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Water-insoluble, pentosanlike material containing uronic acid, arabinose, and xylose residues is present in the outer portion (bran) of the wheat grain. A similar material is present in corn hulls and oat hulls. Within the wheat kernel are found glucose, fructose, maltose, fructosyl-raffinose, a number of glucofructosans (levosine) found also in barley, at least two pentosans (hemicelluloses) composed of arabinose and xylose, and starch, which is the major carbohydrate component of all cereal grains. Wheat germ contains sucrose, raffinose, and traces of glucose and fructose; exposure of wheat kernels to moisture results in a decrease of the concentration of these sugars. Barley and oat grains contain a polyglucosan in which the glucose units are joined by 1,3- and 1,4- linkages.

CEREAL GRAINS form a large and important source of food for both man and animals. It is important, therefore, that the components of the grains, which form the "reactants" of the food technologist, be separated and sub-

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jected to careful study, for only in this way are sustained and far-reaching advances likely to be made.

The physical and chemical behavior of wheat flour, for example, has often been related to either the starch or the protein fractions, which together constitute the major portion of the material. However, it has long been realized that

a number of carbohydrates other than starch are present and may play important roles in the physicochemical properties of the flours. It is principally to these relatively minor carbohydrate components in wheat and other grains that attention has been directed in recent years.

Cereal grains, indeed all plant seeds,