Dough Forming Properties[1]

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ABSTRACT

Although much has been learned about the gluten protein complex in the past 50 years, we still do not know why gluten proteins form a dough. We are left pointing out how gluten differs from other proteins, and offering a few tentative hypotheses.

Wheat flour is unique in its ability to form a dough when wetted with water and mixed. Both water and the application of mechanical energy are necessary to form wheat flour into dough. The dough thus formed has the ability to trap and hold gas produced by panary fermentation and produce the light baked products we are all familiar with.

It is generally believed that the gluten proteins of wheat are responsible for that unique property. The gluten proteins are the storage protein of wheat. They are essentially insoluble in water and are easy to isolate in relatively pure form. Manually working a dough under a small stream of water will remove the starch and water solubles and leave you with a rubbery ball of gluten. Gluten was first isolated in 1728 by Beccari in Italy. His report was the first report of protein being isolated from plant material. Gluten as it is isolated probably is an artifact, that is, it is not formed until it is wetted and mixed. As isolated from flour, gluten contains (on a dry basis) about 80% protein, 8% lipids, with the remainder being ash (or mineral) and a small amount of carbohydrate (1). A comprehensive review of the gluten proteins was recently published (2).

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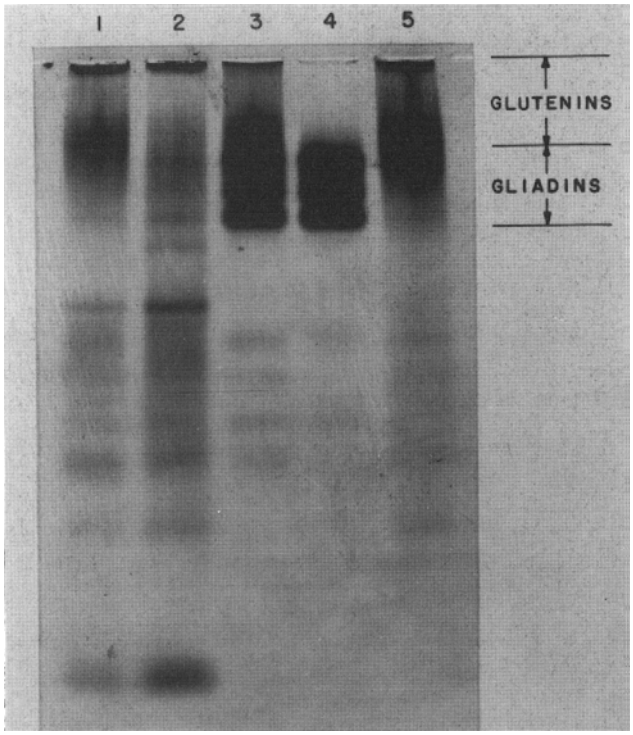

FIG. 1. Starch-gel electrophoretic patterns of protein fractions obtained by ultracentrifugation and solubilization in 70% ethyl alcohol of the pH 4.7 soluble gluten. The patterns represent: insoluble in 70% ethyl alcohol (glutenins, pattern 1), 100-5C glutenin, centrifugate from 100,000 x G for 5 hr. (pattern 2), 100-5S supernatant from soluble gluten after 100,000 x G for 5 hr. (pattern 3), soluble in 70% ethyl alcohol (gliadins, pattern 4), insoluble in 70% ethyl alcohol from 100-5S (100-5S glutenins, pattern 5), Ref. 3.

THE GLUTEN COMPLEX

The gluten complex is composed of two groups of proteins, a prolamine (gliadin) and a glutelin (glutenin). The two groups can conveniently be separated by solubilizing the gluten in dilute lactic acid (pH 4.7), adding ethyl alcohol to make the solution 70% alcohol, and adding sufficient base to neutralize the acid. Upon standing at 4 C, the glutenins will precipitate leaving the gliadin proteins in solution. The gliadins appear to be a rather large group of proteins with similar properties: they have an average molecular weight of about 40,000, are single chained and extremely sticky when hydrated. Those proteins have little or no resistance to extension. The gliadin appears to give the dough its cohesiveness.

The glutenin is also a heterogenous group of proteins that appears to be multichained and varies in molecular weight from 100,000 to several million, with an average of about 3 million. Physically, the protein is resilient, not particularly cohesive, and apparently gives gluten its resistance to extension properties.

Starch gel electrophoresis can be used to characterize the two groups of proteins (Fig. 1). The gliadins are a group of similarly migrating bands. The glutenins do not migrate into the starch gel because they are too large to enter the gel pores. Instead they pack at the surface and form streaks. In free solution they will migrate as one band with a mobility equal to the fastest moving gliadin band (4). The faster moving bands are the water- and salt-soluble proteins and are considered to be contaminants. However, there is no direct evidence that some of these proteins are not involved in the gluten complex.

The heterogeneity of the gliadin fraction is illustrated even better by a combination of isoelectric focusing and starch gel electrophoresis (Fig. 2). By this technique as many as 40 different gliadin proteins have been identified.

Another useful technique, particularly with the glutenin protein, is SDS electrophoresis after breaking the disulfide bonds with mercaptoethanol (Fig. 3). Thus, the glutenin is reduced to single chains that will migrate into the gel according to their molecular weight. The chains vary from

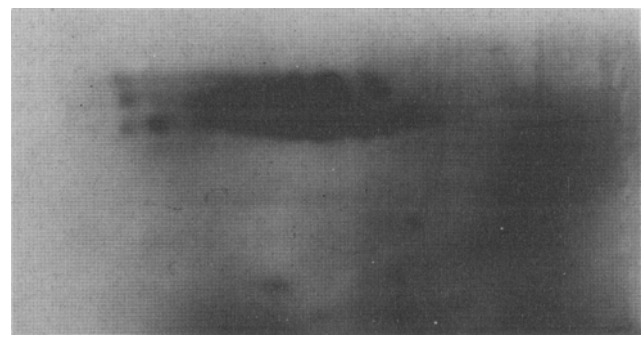

FIG. 2. Two-dimensional characterization of wheat gliadin proteins. First dimension (horizontal) was by gel electrofocusing: pH range 5 to 9. Second dimension (vertical) by starch gel electrophoresis in 3 M urea, aluminum lactate buffer pH 3.2 (Ref. 5).

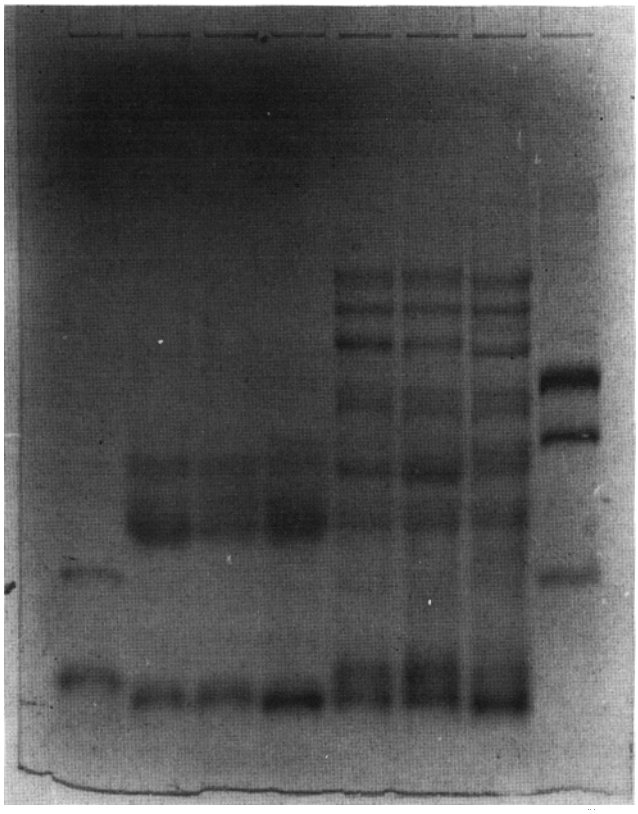

FIG. 3. SDS electrophoresis comparing gliadins and glutenins from three varieties of wheat flours (left to right). Standard proteins (myoglobin and chymotrypsinogen), gliadin-1, gliadin-2, gliadin-3, glutenin-1, glutenin-2, glutenin-3, and standard proteins (bovine serum albumin and chymotrypsinogen), Ref. 5.

less than 16,000 to about 133,000 in molecular weight (7).

We really don't know why gluten proteins form a dough. However, several facts about the gluten proteins are unusual and may be related to their dough forming ability. For example, the gluten proteins are low in basic amino acids (Table I). The low level of lysine is well known. The gluten proteins are very high in glutamic acid (35% of the protein), thus more than 1 of every 3 amino acids in the protein is glutamic acid. The glutamic acid residues in the proteins occur mainly as their amide, glutamine, rather than as free acids. Evidence for the amide is the high ammonia nitrogen found after acid hydrolysis and the fact that gluten protein will not electrophoretically migrate in alkaline buffers. Failure to migrate at any one pH would indicate neutrality. However, failure to migrate over a broad range of pH values is taken as evidence that there are practically no negative charges on the proteins in alkaline media.

The next most notable point about gluten's amino acid composition (Table I) is the high level of proline (14%). Thus, one of each seven amino acid residues is proline. Because proline's amino group is involved in a ring structure, proline causes a kink in the protein chain. The protein cannot readily form into a α-helix. Measurement of helial structure has generally given low values (8). The remaining amino acids have a reasonable amount of amino acids with hydrophobic side chains and relatively low amounts of sulfur containing amino acids.

In general, the amino acid composition shows one-half of the protein made up of two amino acids (glutamine and proline). The charge on the proteins is extremely low, with low levels of basic amino acids and practically all the acidic groups occurring as their amides. With the high amide groups and the low charge on the protein, we might suspect substantial hydrogen bonding in the system. Evidence for the importance of H-bonding is shown by mixing flour with

TABLE I

Amino Acid Composition (Moles/10^5 G.) of Gluten, Gliadin, and Glutenin (Ref. 1)

Amino acid	Gluten	Gliadin	Glutenin
Arginine	20	15	20
Histidine	15	15	13
Lysine	9	5	13
Threonine	21	18	26
Serine	40	38	50
Aspartic acid	22	20	23
Glutamic acid	290	317	278
Glycine	47	25	78
Alanine	30	25	34
Valine	45	43	41
Leucine	59	62	57
Isoleucine	33	37	28
Proline	137	148	114
Tyrosine	20	16	25
Phenylalanine	32	38	27
Tryptophan	6	5	8
Cystine/2	14	10	10
Methionine	12	12	12
Ammonia	298	301	240

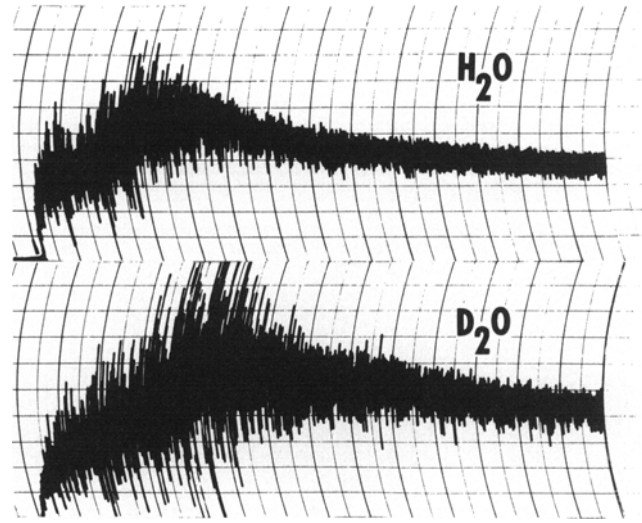

FIG. 4. Mixograms of a flour mixed with water and with D_2O.

TABLE II

Lipid Content of Gluten and Certain of its Fractions (Ref. 10)

Sample	Yield %	Pet. ether Extractable %	Bound lipid %	Total lipid %
Flour		0.80	0.60	1.40
Gluten	100	0.56	5.84	6.40
Gliadin	53	4.29	3.90	8.19
Glutenin	47	1.40	2.97	4.37
100-5C	15	13.47	6.67	20.14
100-5C(Defatted)	15	0.81	3.95	4.76

D_2O instead of H_2O (Fig. 4). The dough produced is much stronger. If we add a hydrogen bond-breading reagent such as urea, the dough is much weaker.

EFFECT OF LIPIDS, pH, MIXING, AND OTHER CONSTITUENTS

The gluten proteins also effectively bind lipids (9). Flour contains about 0.8% lipids that can be extracted with petroleum ether; however, after flour is wetted and mixed into a dough, only about 0.3% of the lipids are extractable. Gluten protein will bind great quantities of lipids.

When isolated by alcoholic fractionation, both the

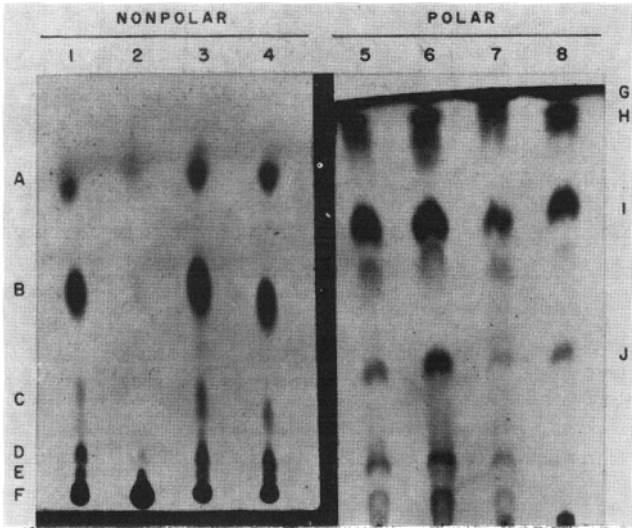

FIG. 5. Thin layer chromatograms of lipids from gluten fractions. Patterns 1 and 5 represent bound lipids extracted from gluten. Patterns 2 and 6 represent bound lipids extracted from gliadins (soluble in 70% ethanol). Patterns 3 and 7 represent bound lipids extracted from glutenin (insoluble in 70% ethanol). Patterns 4 and 8 represent lipids extracted with petroleum ether from 100-5C glutenins. Spots are tentatively identified as follows: A, hydrocarbons and steryl esters; B, triglycerides; C, free fatty acids; D, diglycerides; E, monoglycerides; F, unresolved polar lipids; G, unresolved nonpolar lipids; H, monogalactosyl diglycerides; I, digalactosyl diglycerides; and J, phosphatidyl choline (Ref. 10).

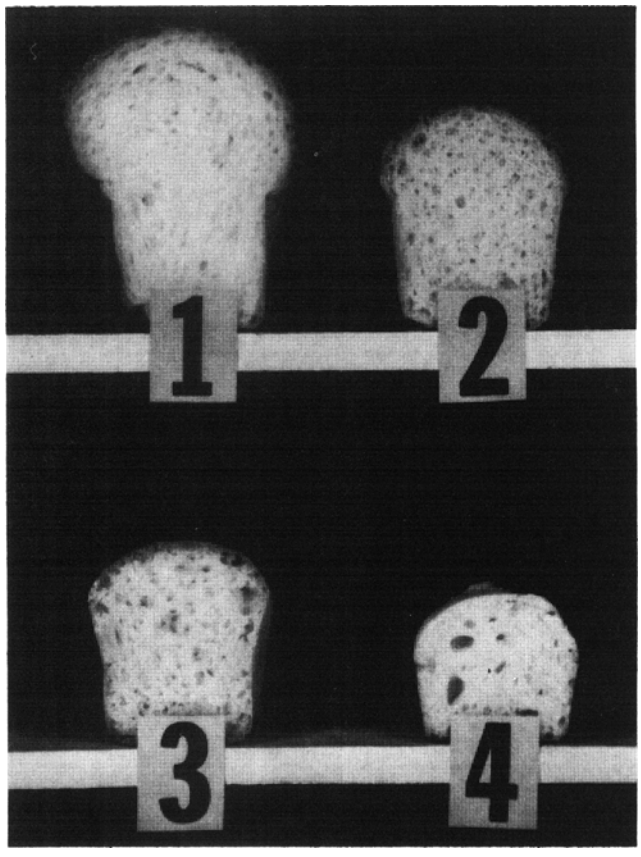

FIG. 6. Cut loaves baked from original flour (1), reconstituted flour containing crude gluten (2), and gluten solubilized in lactic acid at pH 4.53 (3) and pH 3.27 (4), Ref. 12.

gliadin and glutenin retain considerable bound lipid (Table II). The bound lipids in the gliadin fraction are all polar, while lipids bound by the glutenin are both polar and nonpolar (Fig. 5). This is interpreted to mean that the gliadin-

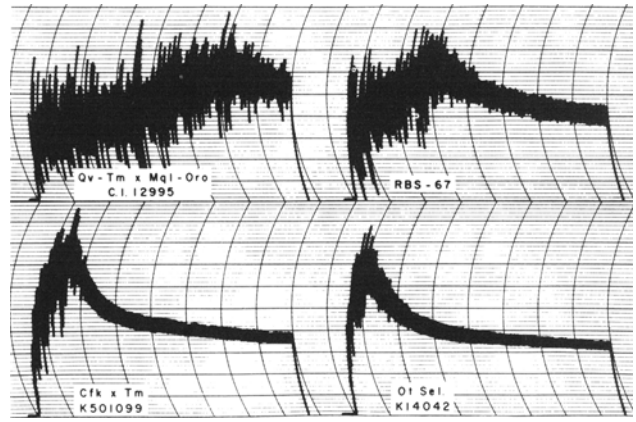

FIG. 7. Mixograms of typical hard winter wheat flours that have excellent (C.I. 12995), good (RBS), poor (K501099), and extremely poor (K14042) mixing properties (Ref. 13).

lipid bond is polar (possibly H-bonding) while the glutenin-lipid bond is hydrophobic (10). NMR data appear to confirm the hydrophobic binding (11). It has been suggested that the gliadin-gluten are bound together by polar lipids (10).

The effect of pH on gluten protein is also quite pronounced (12). The loaves of bread pictured (Fig. 6) were baked after the gluten was taken to different pHs and then neutralized. Exposure of the gluten to pHs below 4 will essentially wreck the ability of the gluten to produce a light loaf of bread. It is not clear what is responsible—possibly a configurational change, or a splitting of amide groups, or the breaking of certain susceptible bonds in the protein chain.

Different flours have different mixing properties, as illustrated in mixogram curves (Fig. 7). As the flour-water mixture is continually mixed, the dough becomes more resistant to extension, giving a stronger curve, until a certain point is reached (point of minimum mobility), then the dough breaks down. An optimum loaf of bread can only be made with a dough mixed to the point of minimum mobility. Several reagents can be used to alter mixing time, presumably by breaking the glutenin protein into smaller units.

Although the gluten proteins control the dough forming properties of the wheat flour system, those properties are modified by certain soluble components (13). The active soluble fraction was shown to be a pentosan-protein fraction or possibly a glycoprotein. That fraction makes the dough more extensible and enhances its ability to retain gas, but the mechanism is essentially unknown.

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