Functional (Bread-Making) Properties of Wheat Protein Fractions Obtained by Ultracentrifugation[1]

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ABSTRACT

Good-quality (RBS-76) and poor-quality (76-412) hard winter wheat flours were fractionated into crude gluten and starch plus water-soluble fractions. Gluten proteins, solubilized in 0.0045–0.005*N* lactic acid, were sedimented by ultracentrifugation into a pellet (high molecular weight, relatively insoluble glutenins), a gel (low molecular weight, relatively soluble glutenins), a viscous layer (high molecular weight, soluble gliadins), and a supernatant (low molecular weight, soluble gliadins). The corresponding gel and viscous layer plus supernatant fractions of the good- and poor-quality flours were interchanged singly in reconstituted flours containing the starch plus water-soluble fraction and baked into bread (10 g of flour). The gel glutenin proteins of the acid-soluble gluten proteins controlled mixing requirement and baking absorption, and the viscous layer and supernatant gliadin proteins controlled loaf volume and crumb grain. Sedimentation rates and physical properties of the different protein fractions varied greatly within a quality level, and the sedimentation rate of a given protein fraction varied materially between the two quality levels. When progressing from the polyacrylamide gel electrophoresis patterns of the supernatant to the gel protein fractions, densities of the rapidly moving bands (small proteins) decreased, and number and densities of the slowly moving bands (large proteins) increased.

Breadmaking, involving biochemical and physical-chemical systems, is an essential analytical test in the scheme of relating physical and biochemical properties of important wheat-flour fractions and components to functional properties. The only apparent way to bridge the gap between functional baking research and biochemical research on wheat and flour is by fractionation and reconstitution techniques. Normal functional properties of wheat fractions and components can be demonstrated only when each fraction is chemically and physically unaltered and is allowed to perform and interact singly and in various combinations with the other essential ingredients of a fermenting and optimum dough (Finney 1954, 1978; Finney et al 1976). Fractions and components are considered to be unaltered when the functional properties of the reconstituted flour or dough are equal to those of the original flour. The success of fractionation and reconstitution techniques in bridging the two areas of research and in identifying the wheat-flour components responsible for specific functional properties was reviewed by Finney (1971) and Hoseney and Finney (1971). Those studies yielded strong evidence that the glutenin proteins were responsible for mixing requirement and the gliadin proteins for loaf volume potential of bread wheat flours. Also, by ultracentrifuging (at 100,000 × *g*) the gluten proteins that were solubilized at pH 4.6 in 0.0005*N* lactic acid, about 32% of the glutenins were sedimented as a pellet (centrifugate, 100-5C). The supernatant (100-5S) contained all of the gliadins and about 68% of the glutenins that were precipitated after making the supernatant 70% ethanol. The glutenins were functional, but the gliadins were not separated as a functional protein fraction. Goforth and Finney (1976) physically separated glutenin from gliadin by ultracentrifuging the acid-soluble gluten at 435,000 × *g*. Properties of bread made from the preliminary reconstitutions of the wet protein fractions indicated that the gliadin and glutenin fractions were 100% functional.[4] Based on those studies, Jones et al[5] found that the corresponding fractions of acid-solubilized glutens of good- and poor-quality wheat flours sedimented at different rates. They determined the time at 100,000 × *g* required to sediment the 11–15% high molecular weight protein fraction (pellet) and the time at 435,000 × *g* to sediment an amount of gel protein (35–31%), so that about 54% of the acid-solubilized gluten protein would remain in the combined viscous layer and supernatant protein fractions of both good- and poor-quality bread wheats.

The gel, viscous layer, and supernatant gluten proteins sedimented at 435,000 × *g* are esentially the same proteins that were unseparated in the 100-5S fraction described in the reviews of Hoseney and Finney (1971) and Finney (1971). Therefore, in the present fractionation and reconstitution studies, we interchanged those corresponding protein fractions of good- and poor-quality wheat flours and related them and their physical and biochemical properties to functional properties.

MATERIALS AND METHODS

Wheat and Flour Samples

Regional baking standard 1976 (RBS-76) was a good-quality composite of many hard red winter wheat varieties harvested throughout the Great Plains in 1975 and 1976. RBS-76 wheat contained 13.4% protein (14% mb) and 1.59% ash. Its straight grade flour contained 12.5% protein and 0.41% ash. A poor-quality wheat (76-412) was a composite of two Chiefkan × Tenmarq progenies (KS501097 and KS501099) from each of the 1974 and 1976 crops. 76-412 wheat contained 13.5% protein and 1.52% ash. Its straight grade flour contained 12.7% protein and 0.49% ash. Analytical data are expressed on a 14% mb.

Analytical Procedures

Moisture, protein, and ash contents were determined by AACC approved methods 44-15A, 46-11, and 08-01, respectively.

The modified, 10-g baking method of Shogren et al (1969) employed optimum mixing, optimum water absorption, optimum ascorbic acid (50 ppm, an excess in the absence of nonfat dry milk), and a formula that included 10 g of flour, 0.6 g of sugar, 0.15 g of salt, 0.3 g of shortening, 0.35 g of yeast, 0.4 of Ardex 550 soy flour, and 0.025 g of barley malt (52 DU/g). Ascorbic acid is its own buffer against overoxidation (Shogren and Finney 1974). Doughs were fermented 2 hr and proofed 49 min (time to proof controls to 7.7 cm) at 30° C. Additional related details are given by Finney (1945), Finney and Barmore (1943, 1945a, 1945b), and Finney et al (1976).

The difference between two treatment means (n = 2) required for significance at $P = 0.05$ was 3.5 cc of loaf volume, 0.9% of absorption, and 0.1 min of mixing time.

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was performed as described by Bietz and Wall (1972). Mixograms were made as described by Finney and Shogren (1972).

[1] Presented at the Sixth International Cereal and Bread Congress, Winnipeg, Canada, September 1978.

[2] Research chemist, research chemist, and research food technologist, respectively.

[3] Reference to a company or product does not imply approval or recommendation of the company or product by the USDA to the exclusion of others that may be suitable.

[4] Goforth and Finney. Unpublished data.

[5] B. L. Jones, K. F. Finney, and G. L. Lookhart. Physical and biochemical properties of wheat protein fractions obtained by ultracentrifugation. Presented at the Sixth International Cereal and Bread Congress, Winnipeg, Canada, September 1978.

Fractionation of Flour into Gluten and Starch Plus Water Solubles

Flour (250 g on a 14% mb, 4° C) was placed in the 5-qt mixing bowl and parted from the center to the wall of the bowl so that a well was formed. After adding 160–200 ml of distilled water (4° C) in the well, depending on flour absorption, bowl contents were mixed at 40 rpm for about 30 sec and then at 120 rpm for 2 min on a Hobart K5-A equipped with the flat beater. The doughs were separated into gluten (82% protein, db) and starch plus water solubles (St/WS) by kneading and washing with one rubber-gloved hand in a series of four 75-ml and six 50-ml aliquots of distilled water (4° C). The starch removed by kneading and the wash water for each aliquot of water was strained through a 32-mesh stainless steel screen to remove any small particles of dough or gluten so that they could be recombined with the dough or gluten mass. The dough was gently kneaded during the first few aliquots of water, especially the first, taking special care to keep the dough intact. The last 50-ml aliquot of wash was relatively clear and not milky in appearance.

The starch and water solubles, accumulated in one 2,000-ml beaker beneath the 32-mesh screen, were shelled by freezing in concentric layers on the inside wall of a 1-gal bottle and then lyophilized. The lyophilized starch plus water-soluble fraction was ground in a mortar to pass a 100-mesh Tyler sieve. The wet crude gluten was allowed to relax at 4° C in a covered jar (5 hr for RBS-76 and 2 hr for 76-412). The wet gluten was lyophilized after freezing in thin (3-mm) monolayers on rectangular aluminum plates that were inserted into wide-mouth bottles. Lyophilized gluten was ground in a micro-Wiley mill to pass a 60-mesh sieve. Each fractionation was carried out as a gravimetric analysis. All fractions, after being analyzed for moisture and protein content, were stored at −20° C. Grams of gluten and starch plus water solubles required to give a reconstituted flour equal in protein content to that of the original flour (14% mb) were calculated from simultaneous equations involving "as received" protein and dry matter contents of the fractions.

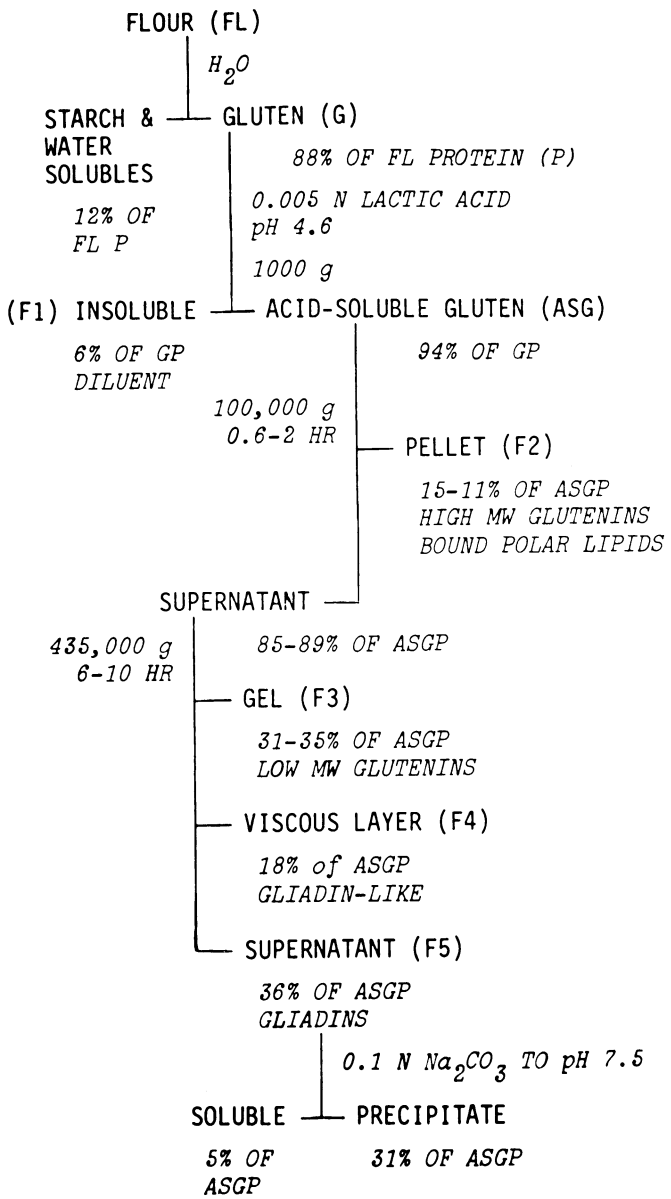

Fig. 1. Scheme to fractionate wheat flour into crude gluten protein and starch plus water solubles and to fractionate the acid-soluble gluten into two glutenin and two gliadin fractions.

Flour of 76-412 was more difficult to fractionate than was RBS-76 flour. Therefore, the crude gluten of 76-412 was somewhat more contaminated with starch than that of RBS-76. The average ratio of crude-gluten yield to St/WS yield of RBS-76 and 76-412 was about 1:5.

Fractionation of Gluten Protein by Ultracentrifugation

About 16 g of lyophilized gluten (14% mb, about 11.3 g of protein) was solubilized in about 335 ml of lactic acid (0.005*N* for RBS-76 and 0.0045*N* for 76-412) in a Waring Blendor for 5 min at low speed. The poor-quality protein needed to be solubilized at about pH 5 instead of pH 4.6 to prevent damage (Goforth et al 1977). Contents of the blender were poured into a 1,000-ml graduated cylinder, where they remained for about 3 hr until foam was eliminated. Contents of the cylinder then were centrifuged at 1,000 × *g* for 20 min (Fig. 1). The acid-soluble gluten (ASG), containing 94% of the gluten protein (GP), was ultracentrifuged at 100,000 × *g* (35 min for 76-412 and 2 hr for RBS-76) to sediment the least soluble, high molecular weight glutenins (F2). Then the supernatant was decanted into eight more 38.5-ml polyallomer tubes and ultracentrifuged at 435,000 × *g* (65,000 rpm) in a 70-titanium rotor for 6 hr (76-412) or 10 hr (RBS-76). The protein in the supernatant (F5) was precipitated by titrating with 0.1*N* sodium carbonate to pH 7.5. GP fractions F1 to F5 (precipitate) were lyophilized to a moisture content of 5–8% and then either pestled through a 60-mesh sieve or ground in a micro-Wiley mill to pass a 60-mesh sieve. All fractionations were carried out quantitatively. All fractions, after being analyzed for moisture and protein content, were stored at −20° C. Fractions were reconstituted with St/WS on the basis of recovery ratios and moisture and protein contents to give a reconstituted flour equal in protein content to that of the original flour (14% mb). An equivalent amount of gel proteins (F3) was substituted for the high molecular weight pellet proteins (F2) to maintain the glutenin content of unfractionated gluten.

RESULTS AND DISCUSSION

Starch and Water-Soluble Fractions of RBS-76 and 76-412

Sometimes the St/WS fraction of a poor-quality wheat flour is somewhat inferior to that of a good-quality flour. Loaf volume (64 cc, Table I) of the reconstituted flour containing 76-412 gluten and

TABLE I
Functional Data for Good-Quality RBS-76 and Poor-Quality 76-412 Reconstituted Flours Containing Crude Gluten and Starch Plus Water-Solubles and for Reconstituted Flour Containing 76-412 Gluten and RBS-76 Starch Plus Water Solubles[a]

Reconstituted Flour	Bake Mix Time (min)	Bake Absorption (%)	Crumb Grain[b]	Loaf Volume (cc)
RBS-76 Control	4	62.7	S	85
RBS Gluten + RBS St/WS	2	63.9	S	86
76-412 Control	1	66.6	U^{1-2}	67
412 Gluten + 412 St/WS	¾	66.1	U^{1-2}	64
412 Gluten + RBS St/WS	¾	60.8	U^{1-2}	68

[a] Starch plus water solubles = St/WS.
[b] S = satisfactory, U^{1-2} = approximately 50% poorer than U, where U = U^1.

St/WS fractions was not significantly below that (67 cc) of unfractionated 76-412. Loaf volume (68 cc) of the reconstituted flour containing 76-412 gluten and the St/WS fraction of RBS-76, however, was fully equal to that (67 cc) of unfractionated 76-412. Also, loaf volume (86 cc) of the reconstituted RBS-76 was fully equal to that (85 cc) of its control. Thus, the St/WS fraction of RBS-76 was used in subsequent reconstituted flours of both 76-412 and RBS-76, in which the gluten protein fractions of the poor- and good-quality flours were interchanged.

Baking absorption (66.6%, Table I) of the poor-quality 76-412 unfractionated control flour was about four percentage points greater than that (62.7%) of the good-quality RBS-76 control flour. Absorption (66.1%) of the reconstituted 76-412 was about the same as that (66.6%) of the 76-412 control. However, when RBS-76 St/WS replaced those of 76-412, absorption decreased more than five percentage points to 60.8%. Thus, absorption (60.8%) of 76-412 gluten and RBS St/WS was about three percentage points lower than that (63.9%) of RBS-76 gluten plus RBS St/WS, and the high absorption (66.6%) of 76-412 unfractionated control flour is attributable to its St/WS fraction.

Role of Functional Glutenin and Gliadin Protein Fractions

The top left and right mixograms (Fig. 2) are for the good-quality RBS-76 composite and poor-quality 76-412 composite unfractionated flours, respectively. The middle left mixogram is for the reconstituted good quality RBS-76 flour containing the gel glutenins (PF3) and the gliadins (PF4 and 5). The time to the peak or point of minimum mobility is 4 min, about equal to that of the control. The middle right mixogram is for the reconstituted poor-quality 76-412 flour containing the gel glutenins and the gliadins. The mixing time of 2 ⅛ min to the point of minimum mobility is similar to that of the 76-412 control. The lower left mixogram is for the reconstituted flour containing the gel glutenins (PF3) of the poor-quality flour and the gliadins (PF4 and 5) of the good-quality flour; it has mixing properties essentially equal to those of the poor-quality reconstituted flour (middle right). The lower right mixogram is for the reconstituted flour containing the gel glutenins of the good-quality flour and the gliadins of the poor-quality flour; and its mixing properties are essentially the same as those of the good-quality reconstituted flour (middle left). The mixing times determined independently during baking (Table II) corroborate the corresponding mixograph mixing times. Thus, the gel glutenins control mixing time.

Baking absorption (63.6%, Table II) of RBS-76 reconstituted flour was 2.1 percentage points greater than that (61.5%) of 76-412 reconstituted flour. When RBS-76 PF4 and RBS-76 PF5 (gliadins) were substituted for those of 76-412, baking absorption (61.4%) was essentially the same as that (61.5%) of the 76-412 reconstituted flour. Thus, the difference (2.1 percentage points) in absorptions of the proteins (PF3, 4, and 5) of RBS-76 and 76-412 is attributable to the gel glutenin (PF3). That conclusion is corroborated by the absorption (62.9%) of the reconstituted flour containing the gel glutenins (PF3) of RBS-76 and the gliadins (PF4 and 5) of 76-412. The crude glutens (Table I) differed by 3.1 percentage points. The absence of the high molecular weight pellet glutenins in the reconstituted flours (Table II) may account for the significant absorption differences (2.2 and 1.4 percentage points) between the gel glutenins being less than 3.1 percentage points.

Loaf 1 (85 cc, Fig. 3, Table II) was baked from the unfractionated RBS-76 good-quality flour. Loaf 2 (92 cc), baked from the reconstituted flour containing the gliadins (PF4 and 5) and gel glutenins (PF3) of RBS-76, is significantly larger than loaf 1, probably because the low molecular weight gel glutenins were superior to the replaced high molecular weight pellet glutenins (F2, Fig. 1). Loaf 3 was baked from the reconstituted flour containing the gel glutenins of the poor-quality 76-412 flour and the gliadins of the good-quality RBS-76 flour. Volume of loaf 3 (89 cc), although somewhat smaller than, is essentially equal to that of loaf 2 for the reconstituted good-quality RBS-76 flour, but dough mixing properties (lower left mixogram, Fig. 2) are similar to those of the reconstituted poor-quality 76-412 flour (second row right mixogram). Thus, loaf 3 essentially has the volume potential of the reconstituted good-quality RBS-76 flour and the mixing requirement of the reconstituted poor-quality 76-412 flour.

Loaf 4 (67 cc, Fig. 3, Table II) was baked from the unfractionated 76-412 poor-quality flour. Loaf 5 (75 cc), baked from the reconstituted flour containing the gliadins (PF4 and 5) and gel glutenins (PF3) of 76-412, is significantly larger than loaf 4, also probably because the low molecular weight gel glutenins were superior to the replaced high molecular weight pellet glutenins (F2, Fig. 1). Loaf 6 was baked from the reconstituted flour containing the gel glutenins of the good-quality RBS-76 flour and the gliadins of the poor-quality 76-412 flour. The volume of the loaf 6 (78 cc), although somewhat larger than, is essentially equal to that of loaf 5 for the reconstituted poor-quality 76-412 flour, but dough mixing properties (lower right mixogram, Fig. 2) are essentially equal to those of the reconstituted good-quality RBS-76 flour. Thus, loaf 6 essentially has the volume potential of the reconstituted poor-quality 76-412 flour (second row left mixogram) and the mixing requirement of the reconstituted good-quality RBS-76 flour.

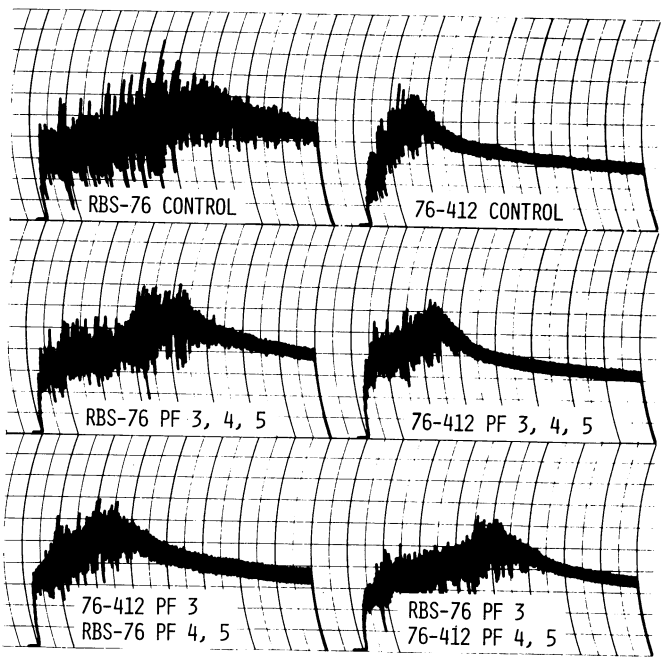

Fig. 2. Mixograms of good (RBS-76) and poor (76-412) bread-making flours (10 g). **Top row**, unfractionated controls. **Middle row**, reconstituted control flours containing the gel low molecular weight glutenins (PF3), the viscous layer high molecular weight gliadins (PF4), the supernatant low molecular weight gliadins (PF5), and RBS starch plus water-solubles. **Bottom row**, mixograms of reconstituted flours in which the glutenins (or gliadins) of RBS-76 and 76-412 were interchanged.

TABLE II
Functional Data for Good-Quality RBS-76 and Poor-Quality 76-412 Reconstituted Flours Containing the Gel, Low Molecular Weight Glutenins (PF3), the Viscous Layer, High Molecular Weight Gliadins (PF4), the Supernatant, Low Molecular Weight Gliadins (PF5), and RBS Starch plus Water Solubles and for Reconstituted Flours in Which the Glutenins (or Gliadins) of RBS-76 and 76-412 Were Interchanged

Reconstituted Flour	Bake Mix Time (min)	Bake Absorption (%)	Crumb Grain[a]	Loaf Volume (cc)
RBS-76 Control	4	62.7	S	85
76-412 Control	1	66.6	U^{1-2}	67
RBS PF3, 4, 5	$3\frac{1}{2}$	63.6	S	92
412 PF3, 4, 5	2	61.5	Q-U	75
412 PF3 + RBS PF4, 5	$2\frac{1}{4}$	61.4	S	89
RBS PF3 + 412 PF4, 5	$3\frac{5}{8}$	62.9	Q-U	78

[a]S = satisfactory, Q = questionable, U = unsatisfactory, U^{1-2} = approximately 50% poorer than U, where U = U^{1}.

The gliadin and glutenin fractions were not pure separations, as would be expected in sedimentation studies. The gel glutenins of 76-412 in loaf 3 carried with them small amounts of the poor-quality gliadins, thereby accounting for loaf 3 being somewhat smaller than loaf 2. Also, the good-quality RBS-76 gel glutenins in loaf 6 contained small amounts of the good-quality gliadins, thereby accounting for loaf 6 being somewhat larger than loaf 5. Nevertheless, the data clearly show that the gel glutenin proteins of the acid-soluble gluten proteins control mixing time and bake absorption, and the gliadin proteins control loaf volume and crumb grain.

PAGE Patterns of Functional Gluten Protein Fractions

The SDS-PAGE patterns (Fig. 4) of the supernatants (F5, Fig. 1) of both flours are distinctly different from those of the VL and the gels. Patterns of the VL and gel protein fractions are qualitatively similar but quantitatively different. As we progress from supernatant to gel, densities of the rapidly moving bands (small proteins) decrease, and number and densities of the slowly moving bands (large proteins) increase. The high molecular weight proteins in the gel protein fractions are absent in the supernatant fractions. The very high molecular weight pellet fractions (F2, Fig. 1) of both RBS-76 and 76-412 gluten proteins were materially different from any of the other gluten protein fractions. Most of the pellet proteins were too large to enter and move in the acrylamide gel. Those that did move probably were trapped in the small amount of liquid absorbed by the sedimented pellet proteins.

The PAGE patterns of comparable protein fractions of the good- and poor-quality flours differ somewhat. For example, the bands about halfway down from the origins of the supernatant, viscous layer, and gel patterns of 76-412 are nearly absent in the corresponding patterns of RBS-76. Also, the bands just above the nearly absent ones are absent in the corresponding patterns of 76-412. Other small differences exist in the slowly moving and the dense, rapidly moving bands of the viscous layer and gel patterns of 76-412 and RBS-76.

Gluten Protein Fractions are Functional Entities

The high molecular weight glutenin proteins sedimented at 100,000 × *g* to give a dense, brown, rubbery, relatively insoluble pellet (35 min for the poor-quality 76-412 flour; 2 hr for the good-quality RBS-76 flour). The low molecular weight glutenin proteins sedimented at 435,000 × *g* to give an off-white, relatively soluble, mesomorphic layer or gel (6 hr for 76-412 flour and 10 hr for RBS-76 flour). The high molecular weight gliadin proteins also sedimented at 435,000 × *g* to give a clear, colorless, viscous solution (6 hr for 76-412 flour and 10 hr for RBS-76 flour). The low molecular weight, very soluble gliadins of both flours remained in the corresponding supernatants. Thus, the sedimentation rates of the different protein fractions varied greatly within a quality level; and the sedimentation rate of a given protein fraction varied materially between the two quality levels. In addition, the pellet, gel, and viscous-layer fractions varied greatly in density, solubility, color, and appearance.

Progressing from the PAGE patterns of the supernatant to the gel protein fractions shows that densities of the rapidly moving bands (small proteins) decreased, and those of the slowly moving bands (large proteins) increased. The high molecular weight proteins in the gel protein fractions of both flours were absent in the supernatant fractions. Most of the high molecular weight pellet glutenins were too large to enter and move in the acrylamide gel.

The foregoing physical and biochemical data and discussion, together with functional data relating the glutenin proteins to mixing requirement and bake absorption and the gliadin proteins to loaf volume and crumb grain, demonstrate that the gluten protein fractions separated by ultracentrifugation are functional entities.

Other Considerations

In SDS-PAGE studies, many types of bonds are broken, so that many component parts of the original functional protein fraction are visible. Those parts likely are irreversibly nonfunctional and may be common to two or more protein fractions that have different physical and functional properties. Thus, the repetition of

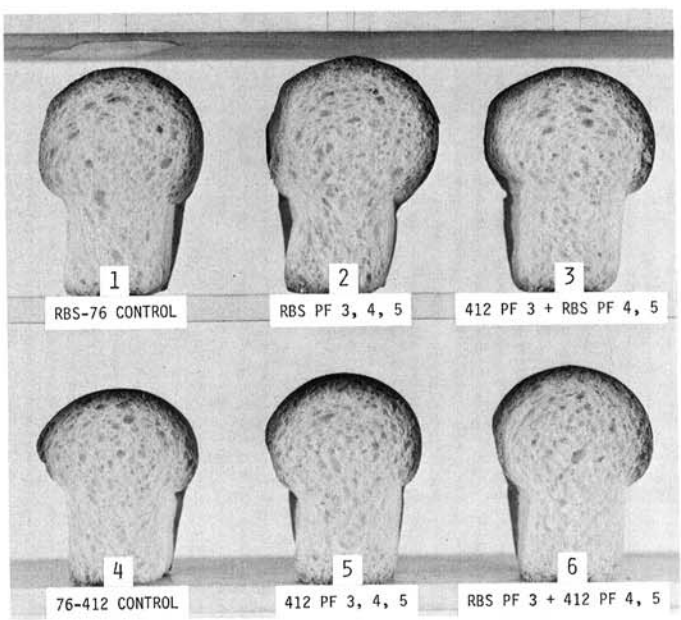

Fig. 3. Cut loaves of bread made from good (RBS-76) and poor (76-412) unfractionated and reconstituted bread-making flours (10 g). 1 (RBS) and 4 (412) are from unfractionated controls. 2 (RBS) and 5 (412) are from reconstituted control flours containing the gel low molecular weight glutenins (PF3), the viscous layer high molecular weight gliadins (PF4), the supernatant low molecular weight gliadins (PF5), and RBS starch plus water-solubles. 3 and 6 are from reconstituted flours in which the glutenins (or gliadins) of RBS-76 and 76-412 were interchanged.

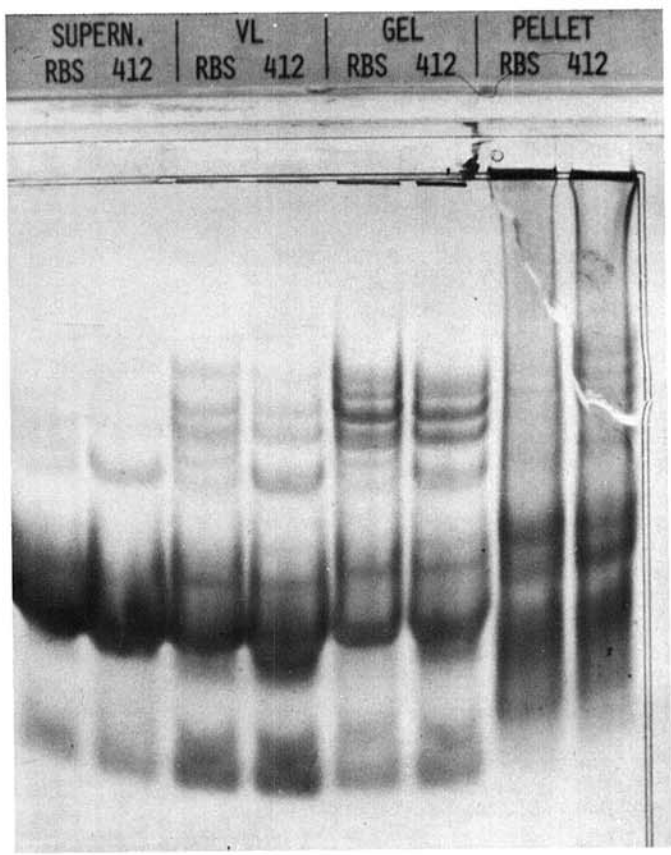

Fig. 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of the supernatant (SUPERN.), viscous layer (VL), gel, and pellet protein fractions ultracentrifuged from acid-soluble glutens of RBS-76 (RBS) good and 76-412 (412) poor bread-making flours.

bands is not necessarily an index of a mixture, impurity, or similarity of proteins.

The relative ease with which the high molecular weight pellet glutenins sedimented after only 35–120 min at only 100,000 × *g* suggests that they are relatively free compared to the low molecular weight gel glutenins that sedimented after an additional 6–10 hr at the high relative centrifugal force of 435,000 × *g*. The gel glutenins appear to be relatively tenaciously interacted or bound to the gliadin proteins. Hoseney et al (1970) demonstrated that essentially all natively bound lipids of wheat flour were polar lipids and were associated with the high molecular weight pellet proteins. The extent to which the bound polar lipids interacted with the pellet proteins, both intramolecularly and intermolecularly to produce very high molecular weight aggregates, may render them relatively nonreactive with the gel glutenin and gliadin proteins, so that the pellet glutenin proteins are relatively free to sediment at relatively low centrifugal forces.

When flour is wetted and mixed into a dough or when gluten is forming, all of the free polar and about half of the free nonpolar lipids become bound (no longer extractable in petroleum ether) and are presumed to form lipoprotein complexes with the glutenin fraction of gluten protein (Olcott and Mecham 1947). The ease of sedimenting the pellet glutenins indicates that they are not involved in the formation of additional lipoprotein complexes and that the free lipids become bound, probably by interacting with reactive gel glutenin and reactive gliadin proteins. Similarly, when the dough is formed, reactive gel glutenins probably interact with reactive gliadins. Thereby, the gel glutenin proteins become bound. When the centrifugation forces are greater than the protein interaction forces but less than the molecular forces that keep the relatively small gliadin proteins in solution, then the gel glutenins sediment.

A variety with a very short (very poor) mixing requirement almost invariably has a low (poor) loaf volume potential. Similarly, a variety with a medium (good) to medium-long (very good) mixing requirement almost invariably has a good to very good (relatively high) loaf volume potential (Finney and Yamazaki 1967). Because loaf volume is a function of gliadin quality, and mixing requirement is a function of glutenin quality, good- and poor-quality glutenins are related to or associated with good- and poor-quality gliadins, respectively. Those relationships are diagrammed in Fig. 5. Finney and Yamazaki (1967) demonstrated that loaf volume increased as mixing requirement increased from less than 1 to about 3 min. Thereafter, loaf volume remained constant for mixing times greater than about 3 min. Those data and similar correlation studies by others have been interpreted by some as evidence that the glutenins control loaf volume. The significant correlation of mixing time and loaf volume is attributable to the probable relationship of glutenin and gliadin quality. One variable being correlated to another does not mean that the one is directly related to or governed by the other. The reconstituted flours in which glutenin or gliadin protein fractions of good- and poor-quality wheat flours were interchanged is direct evidence of the role of each protein fraction in breadmaking.

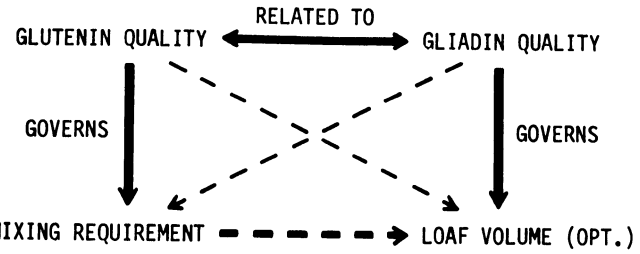

Fig. 5. Diagram of likely direct and indirect relationships between glutenin quality, gliadin quality, and the functional properties, mixing requirement and loaf volume (optimum). Broken lines indicate indirect relationships. The wider the line, the stronger the relationship. Also (not shown), glutenin quality governs mixing tolerance and dough absorption (attributable to flour proteins). Oxidation requirement and dough stability are strongly related indirectly to mixing requirement.

Functional gluten protein fractions must be studied before they are disassembled during physicochemical, biochemical or instrumental analyses, so that PAGE and other biochemical properties can be related to them. The less complex the functional protein fraction, the more specific is the scientific information on that fraction. Thus, studies on functional glutenin fractions and gliadin fractions are more revealing than those on the total functional glutenins or gliadins. Further fractionation of the pellet and gel glutenins and the viscous layer and supernatant gliadins into less complex functional fractions should be additionally revealing, so that PAGE and other biochemical properties can be more specifically related to physicochemical and functional (bread-making) properties.

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