Physical and Biochemical Properties of Wheat Protein Fractions Obtained by Ultracentrifugation[1,2]

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ABSTRACT

Gluten proteins were hand-washed from hard winter wheat flours of good (RBS-76) and poor (76-412) bread-making quality, solubilized in 0.0045–0.0050*N* lactic acid, and separated into four fractions by ultracentrifugation. The four fractions were a brown rubbery pellet, an off-white gel, a colorless viscous layer, and a clear supernatant. The relative sedimentation rates and yields of the fractions were studied. Centrifugation conditions were adjusted so that the ratio of protein in the combined pellet and gel fractions to that in the combined viscous layer and supernatant fractions was about 48:52 for both flours. The sedimentation rates of the different fractions varied greatly within each flour. The sedimentation rates of the fractions differed materially between the two flours. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns showed that the average size of the proteins in each of the four fractions increased progressively from the supernatant to the pellet fractions. Removing the total free lipids from the flours before washing out and dissolving the glutens increased the sedimentation rate of the pellet fraction of 76-412 gluten, but did not affect that of any of the RBS-76 gluten fractions.

Fractionation and reconstitution studies reviewed by Finney (1971) and Hoseney and Finney (1971) provided strong evidence that the glutenin and gliadin proteins were responsible for the mixing requirement and the loaf volume potential, respectively, of bread wheat flours. After the gluten proteins solubilized in 0.0050*N* lactic acid (pH 4.6) were ultracentrifuged at 100,000 × *g* for 5 hr, 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 precipitated after the supernatant was made to contain 70% ethanol. The precipitated glutenins were functional, but the gliadins were not. Goforth and Finney (1976) physically separated glutenin from gliadin by ultracentrifuging the acid-soluble gluten at 435,000 × *g*. Properties of bread made from preliminary reconstitutions of the wet protein fractions separated by ultracentrifugation indicated that the gliadin and glutenin fractions were 100% functional (Goforth and Finney, unpublished data). Based on those studies, we quantitatively separated a supernatant fraction similar to the 100-5S fraction into gel, viscous layer, and supernatant gluten protein fractions by ultracentrifugation at 435,000 × *g*. All fractions retained their bread-making functionality. The functional properties of fractions from good- and poor-quality wheat varieties were described by Finney et al (1982).

We report the time of centrifugation at 100,000 × *g* required to sediment the high molecular weight pellet protein fraction, and the time at 435,000 × *g* needed to sediment an amount of gel protein so that about 54% of the acid-soluble gluten protein remained in the combined viscous layer and supernatant protein fractions of each bread wheat flour. After total free lipids were extracted from the two flours, glutens were prepared, solubilized, centrifuged, and the sedimentation rates of the corresponding fractions were determined. The gel electrophoretic properties of the separated protein fractions from the nondefatted flours are also reported.

MATERIALS AND METHODS

Wheat and Flour Samples

Regional baking standard 1976 (RBS-76) was a good-quality flour milled from a composite of many hard red winter wheat varieties harvested throughout the Great Plains in 1975 and 1976. The RBS-76 wheat composite contained 13.4% protein and 1.59% ash. Its straight grade flour contained 12.5% protein and 0.41% ash. The poor-quality wheat sample (76-412) was a composite of two progenies of Chiefkan × Tenmarq (KS501097 and KS501099) from both the 1974 and 1976 crops. The 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% moisture basis (mb).

Analytical Procedures

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

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed as described by Bietz and Wall (1972). The fractionation of flour into gluten and starch plus water solubles was described in detail by Finney et al (1982).

Fractionation of Gluten Protein by Ultracentrifugation

About 16 g of lyophilized gluten (14% mb, about 11.3 g protein) was solubilized in 335 ml of lactic acid (0.0050*N* for RBS-76 and 0.0045*N* for 76-412) in a Waring blender for 5 min at low speed. The poor-quality protein was solubilized at about pH 5 instead of pH 4.6 so that the loaf volume potential would not be irreversibly damaged (Goforth et al 1977). The blender contents were poured into a 1,000-ml graduated cylinder and remained there until the foam subsided about 3 hr later. The suspension was then centrifuged at 1,000 × *g* for 20 min (Fig. 1). The acid-soluble gluten (ASG)—the supernatant of the 1,000 × *g* centrifugation—which contained 94% of the total gluten protein (GP), was first ultracentrifuged on a Beckman model L2-75B for 2–18 hr at 435,000 × *g* to enable us to identify the various protein fractions and study their relative sedimentation rates and yields over a range of centrifugation times. After each centrifugation, the contents of the tubes were separated into supernatant, viscous layer (VL), gel, and pellet fractions, and the protein content of each was determined.

The pellet material (Figs. 1 and 2) was most efficiently separated from the gel by centrifugation at 100,000 × *g*. Preliminary experiments (centrifugations at 435,000 × *g*) showed that the pellet fraction comprised 11% of the ASGP of RBS-76 and about 15% of the ASGP of 76-412. So that the 100,000 × *g* centrifugation times needed to sediment the pellets could be determined, the ASG of RBS-76 was centrifuged 15–180 min, and that of 76-412 10–80 min.

After centrifuging the ASGP of RBS-76 at 100,000 × *g* for 2 hr and the ASGP of 76-412 for 35 min, the eight supernatants (the rotor held eight tubes) were decanted from their pellets into a second set of eight 38.5-ml polyallomer tubes and ultracentrifuged at 435,000 × *g* (65,000 rpm) in a 70-titanium rotor for various periods of time up to 32 hr. Thus, the sedimentation rates of the gel-protein fractions and the relative amounts of protein in the combined supernatant and VL fractions were determined. Fractions F1 to F5 (Fig. 1) were lyophilized and stored at −20°C

[1] Presented at the Sixth International Cereal and Bread Congress, Winnipeg, Canada, September 1978.
[2] Mention of firm names or trade products does not constitute endorsement by the USDA over others not mentioned.
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until used for reconstitution experiments (Finney et al 1982).

Total free lipids were removed from the flours by refluxing with petroleum ether by the method of Chung et al (1977).

RESULTS AND DISCUSSION

Fractionation of ASGP by Centrifugation

When the ASGP from 76-412 was centrifuged for 2–18 hr at 435,000 × *g* without a preliminary centrifugation at 100,000 × *g*, the pellet formed at the bottom of the centrifuge tube was overlaid with a gel layer that was covered first with the viscous layer and then with the supernatant (Fig. 2, right tube, except that the pellet was beneath the gel fraction). Figure 3 shows how the distribution of protein in the layers changed over time. Essentially all the pellet protein sedimented in less than 2 hr at 435,000 × *g*. Most of the remaining protein moved from the supernatant into the viscous layer, and from there into the gel fraction. Protein in the gel fraction increased from about 23% of the total ASGP after 2 hr of centrifugation to 42% after 18 hr. The protein in the VL increased for about 15 hr and then decreased. The amount of protein in the supernatant decreased rapidly at first and then progressively more slowly. After the supernatant ASGP content reached 15%, further depletion occurred very slowly. The ASGP from RBS-76 behaved similarly.

The amount of protein in the pellet fraction of the acid-soluble

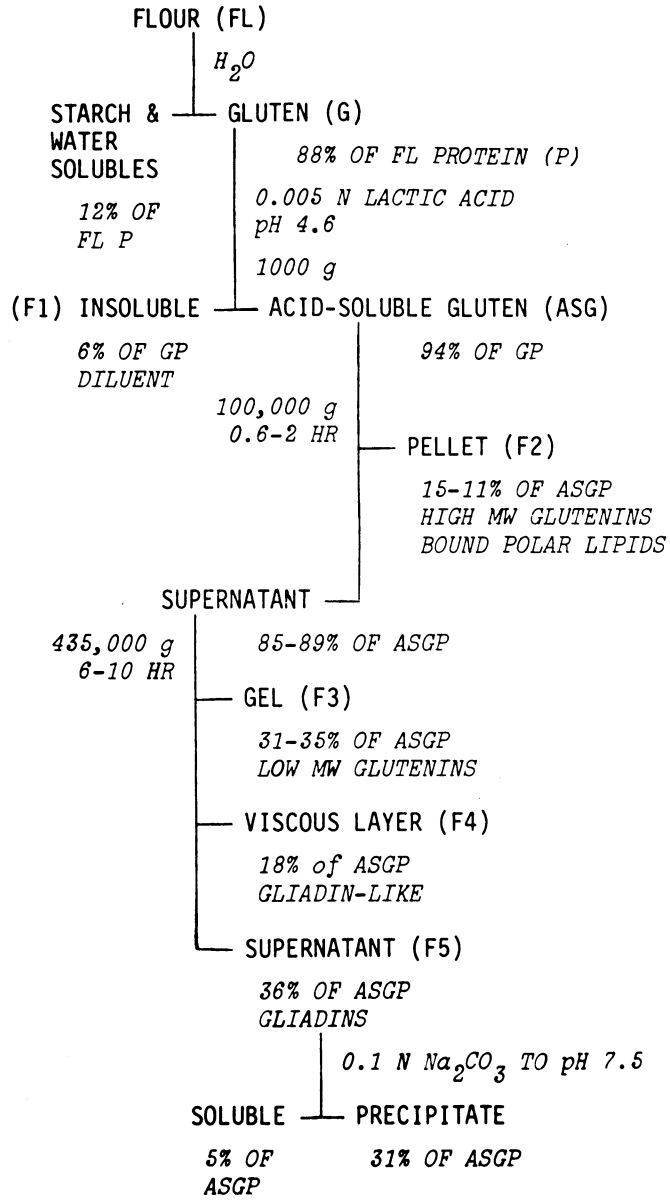

Fig. 1. Methods used to fractionate wheat flour. Hand-washing was used to separate starch and water solubles from the crude gluten. After solubilization in lactic acid, the gluten was separated into four fractions by ultracentrifugation. Fractions F2 and F3 are considered to contain many glutenins; F4 and F5 are considered to contain gliadins.

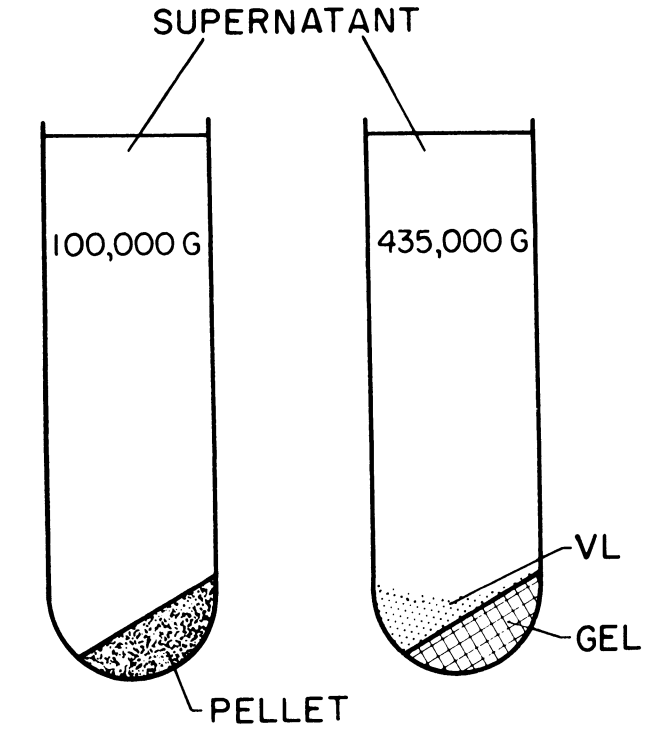

Fig. 2. Diagram of centrifuge tubes showing separated fractions. Centrifugation of solubilized gluten at 100,000 × *g* yielded a pellet and supernatant **(left).** Recentrifugation of the 100,000 × *g* supernatant at 435,000 × *g* resulted in gel, viscous layer (VL), and supernatant fractions **(right).**

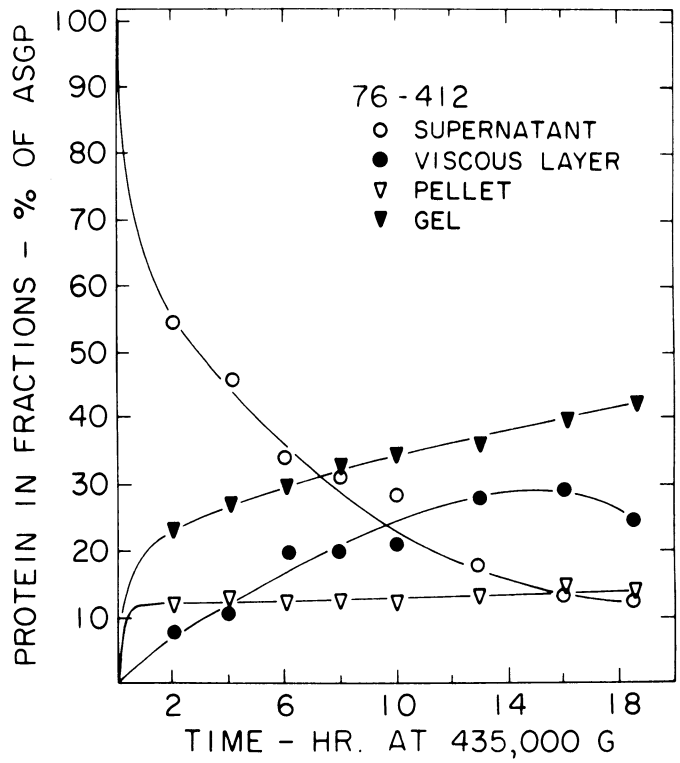

Fig. 3. Effect of centrifugation time at 435,000 × *g* on the distribution of 76-412 acid soluble gluten proteins among various fractions.

76-412 protein increased very little after 2 hr and approached 15% after 18 hr. The pellet protein of RBS-76 gluten behaved similarly in an identical experiment, except that the amount of pellet material remained constant at about 11% of the ASGP instead of about 15%.

Protein in Pellet vs Centrifugation Time

When the dissolved gluten protein from RBS-76 was centrifuged at 100,000 × *g*, the pellet material sedimented in about 120 min (Fig. 4). After the pellet was removed, little more protein sedimented at 100,000 × *g*. The gel protein from RBS-76 gluten was either so relatively small or light (interacted with lipids and gliadins) that it did not sediment at 100,000 × *g*.

The results were different when the gluten proteins of 76-412 were centrifuged at 100,000 × *g* (Fig. 4). The pellet fraction (about 15% of ASGP) sedimented in only 35 min. An additional 8% of the ASGP (total 23%) had sedimented after 90 min. The additional protein layered over the brown pellet was pale yellow or off-white and looked like the gel protein previously described. Thus, some of the gel proteins of 76-412, like the pellet proteins, were sedimented by much lower gravitational forces than were those of RBS-76.

The results suggest that the pellet protein fraction of the poor-quality flour may contain larger molecules or aggregates of molecules than did that of the good-quality gluten. Possibly, the glutenin-lipid-gliadin interactions are relatively weak for the poor-quality 76-412 flour in the lactic acid solution. If that is true, then the 76-412 glutenins would not be held in solution and would be relatively free to form rapidly sedimenting molecular aggregates. Alternatively, the glutenin-glutenin bonding may be stronger in the 76-412 flour so that larger glutenin complexes are formed. In any event, the sedimentation differences may reflect differences in the strengths of protein interactions.

During the 100,000 × *g* centrifugations, much more free lipid-like material was released from the 76-412 solubilized gluten than from the RBS-76 solubilized gluten.

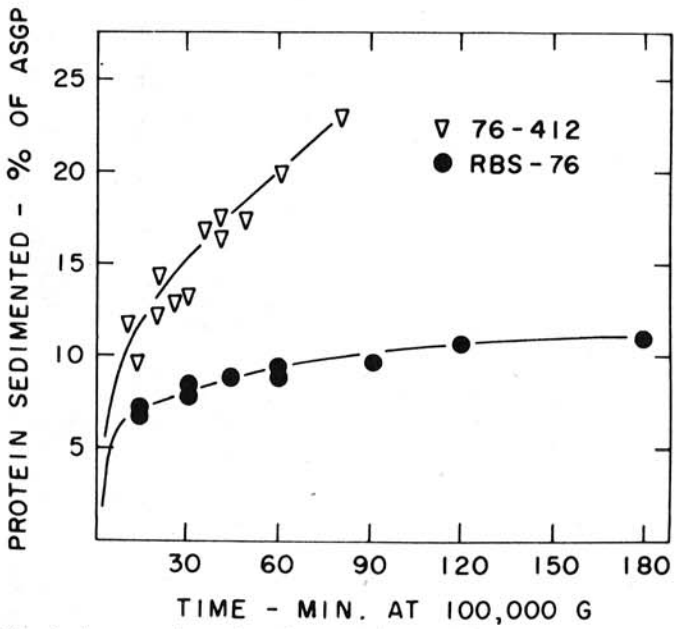

Fig. 4. Amount of protein sedimented from acid-solubilized glutens of good-quality (RBS-76) and poor-quality (76-412) wheat flours vs time of centrifugation at 100,000 × *g*.

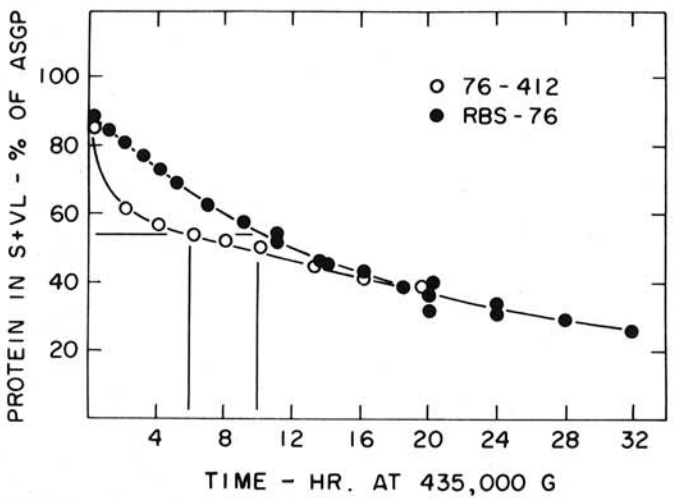

Fig. 5. Amount of protein in the combined supernatant (S) and viscous-layer (VL) fractions of RBS-76 and 76-412 separated from the acid solubilized glutens as a function of time of centrifugation at 435,000 × *g*. The pellet fraction of each gluten was removed by centrifugation at 100,000 × *g* before the 435,000 × *g* centrifugation.

Protein in Supernatant Plus VL vs Centrifugation Time

After the ASGP of RBS-76 was centrifuged for 2 hr and the ASGP of 76-412 for 35 min at 100,000 × *g*, each supernatant was decanted from its pellet and centrifuged at 435,000 × *g* for various periods up to 32 hr (Fig. 5). The gel proteins of 76-412 sedimented more rapidly during the first 2 hr than did those of RBS-76. After 2 hr, the proteins of 76-412 sedimented more slowly than did those of RBS-76, and after 20 hr of centrifugation, equal amounts of protein remained in their combined supernatant and VL fractions.

Because the capacity of the centrifuge rotor is limited to eight tubes and because each point represents the average of duplicate

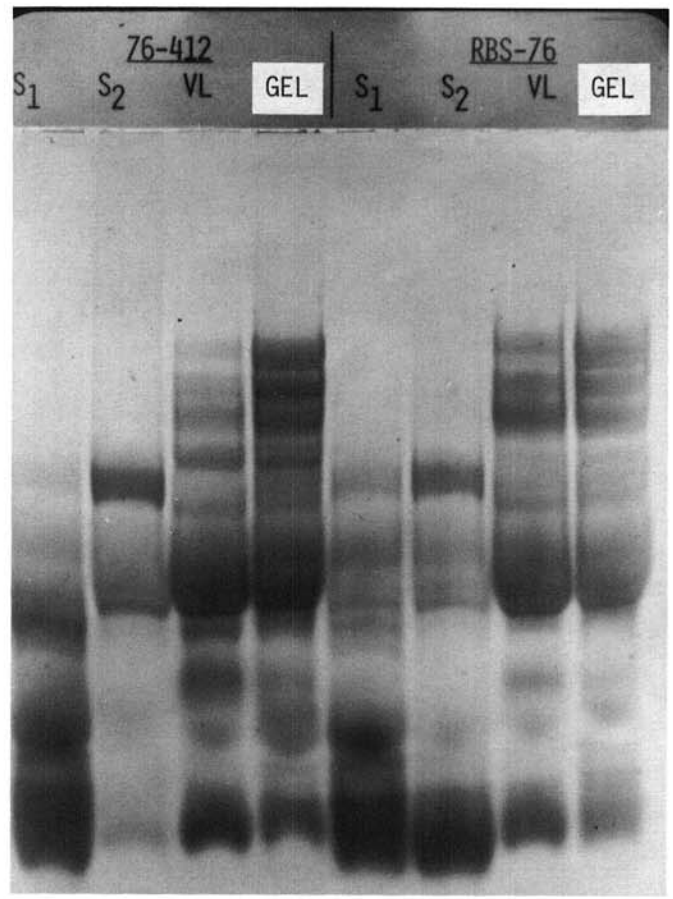

Fig. 6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of proteins from various fractions, separated by ultracentrifugation, of acid-soluble glutens from RBS-76 (good baking quality) and 76-412 (poor baking quality) flours. Fractions were freeze-dried and collected as follows: S_1, top three quarters of supernatant; S_2, bottom quarter of supernatant; VL = viscous layer.

determinations, Fig. 5 is a compilation of data collected from five different ultracentrifugations. All the points (except one at 20 hr) fall on a uniform curve. Thus, the separations obtained by the method are very reproducible.

Before centrifugation at 435,000 × *g*, the supernatant of RBS-76 (after 2 hr at 100,000 × *g*) contained 89% of the ASGP (Fig. 5). After about 10 hr of centrifugation at 435,000 × *g*, 54% of the ASGP remained in the combined supernatant and VL fractions (Fig. 1). Thus, 35% (89 minus 54%) of the protein had sedimented as a gel fraction (slowly sedimenting glutenins). The combined gel (35%) and pellet fractions (11%) contained 46% of the RBS-76 ASGP. Hoseney et al (1969b) and Finney et al (1982) identified those two fractions as glutenin proteins. About 5% of the total ASGP remained in solution after the supernatant was neutralized. Thus, the supernatant proteins insoluble at neutral pH plus the VL fraction contained 49% (54 minus 5%) of the ASGP. If the gluten proteins in the F5 and VL fractions were gliadins, then the gliadin-glutenin ratio was 49:46. This ratio was used in reconstitution studies (Finney et al 1982) and approximates that reported by Hoseney et al (1969a).

Similarly, the 76-412 supernatant (after centrifuging for 35 min at 100,000 × *g*) contained 85% of the ASGP (zero time, Fig. 5). After 6 hr of centrifugation at 435,000 × *g*, approximately 54% of the ASGP remained in the combined supernatant and VL fractions. Thus, 31% (85 minus 54%) of the ASGP had sedimented as a gel fraction (slowly sedimenting glutenins). The gel fraction (31%) plus the pellet fraction (15% of ASGP, rapidly sedimenting glutenins) comprised 46% of the 76-412 ASGP as total glutenin proteins. About 5% of the total ASGP remained in solution after the supernatant was neutralized, so that the combined supernatant and VL fractions of 76-412 also contained 49% (54 minus 5%) of the ASGP. Hoseney et al (1969a) reported that both good and poor varieties had a relatively constant ratio of gliadin to glutenin.

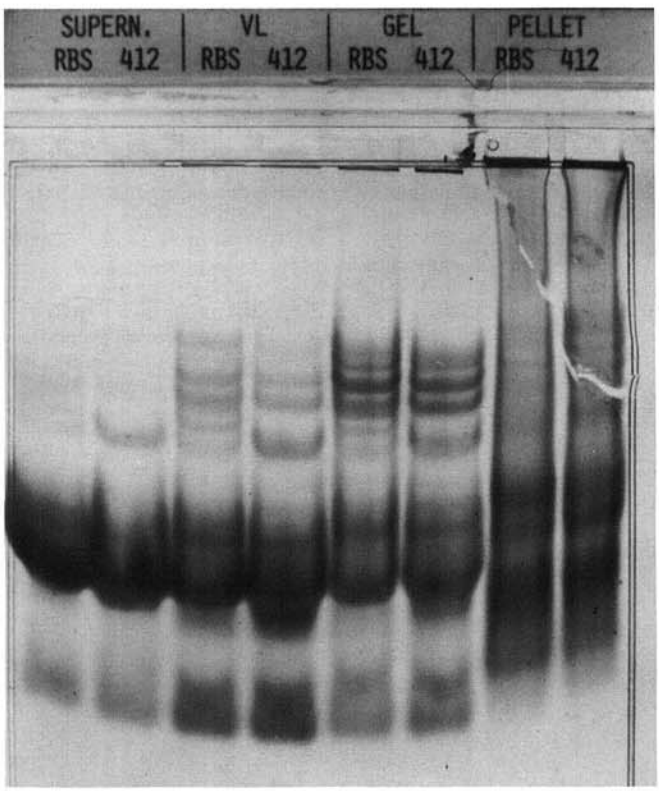

Fig. 7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of proteins from various ultracentrifugally separated fractions of acid soluble glutens from RBS-76 (good baking quality) and 76-412 (poor baking quality) flours. Fractions were freeze-dried before analysis. VL = viscous layer.

PAGE Patterns of Gluten Protein Fractions

The SDS-PAGE patterns (Fig. 6) of the top three quarters (S_1) and the bottom quarter (S_2) of the supernatants (Fig. 1, F5) of RBS-76 and 76-412 each differed greatly and were very distinct from those of their viscous layers and the gels. The patterns of the VL and gel protein fractions were qualitatively similar but quantitatively different. Progressing from supernatant S_1 to gel, the staining densities of the rapidly moving bands (small proteins) decreased, and the number and densities of the slowly moving bands (large proteins) increased. The high molecular weight proteins in the gel protein fractions were not present in the supernatant fractions.

Similarly, the SDS-PAGE patterns (Fig. 7) of the total supernatant fractions of each flour were distinct from those of the viscous layers and the gels. Patterns of the VL and gel protein fractions were qualitatively similar but quantitatively different. Progressing from supernatant to gel, densities of the rapidly moving bands (smaller proteins) decreased, and the number and densities of the slowly moving bands (larger proteins) increased. The high molecular weight proteins in the gel protein fractions were not present in the supernatant fractions. The very high molecular weight pellet fractions of both RBS-76 and 76-412 gluten proteins were materially different from any of the other gluten protein fractions. Much of the pellet protein was too large to enter the acrylamide gel. Protein that did move into the gel may have been trapped in the small amount of solvent associated with the pellet proteins after ultracentrifugation.

Effect of Defatting on Sedimentation of Proteins

Removing the total free lipids from flour before washing out the gluten affected the sedimentation properties of the ASGP of the 76-412 flour (Fig. 8). The time needed to pellet 15% of the ASGP at 100,000 × *g* was reduced from 30 to 35 min (nondefatted) to about 6 min (defatted). Defatting increased the amount of protein sedimented in 35 min from about 15 to 19%. The additional protein sedimented was gel material (low molecular weight glutenins). Because no free lipids were available to form glutenin-lipid-gliadin

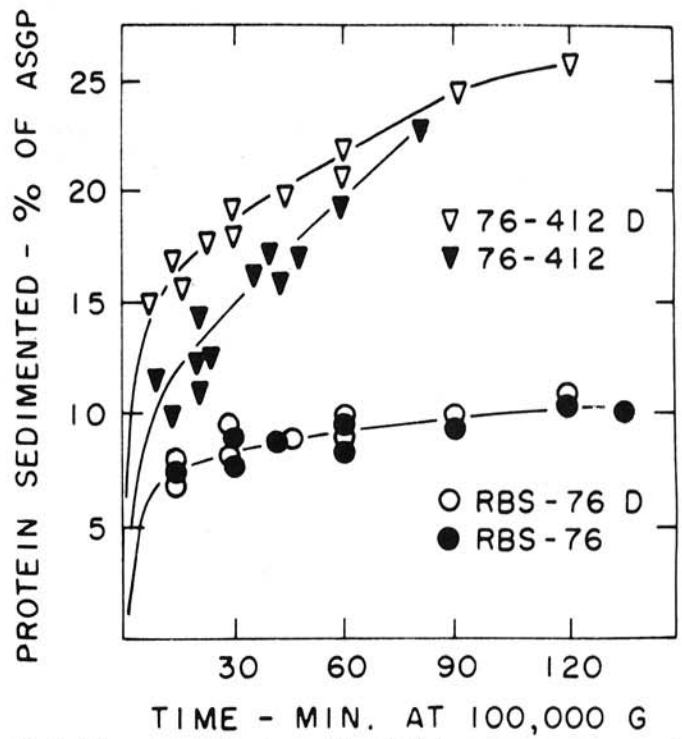

Fig. 8. Effect of removing the total free lipids from flours on the rates of sedimentation of their acid solubilized gluten proteins. Centrifugation was at 100,000 × *g* for periods of 7–135 min. RBS-76 D and 76-412 D flours were identical to RBS-76 and 76-412 flours, except that they were defatted with petroleum ether before their glutens were washed out.

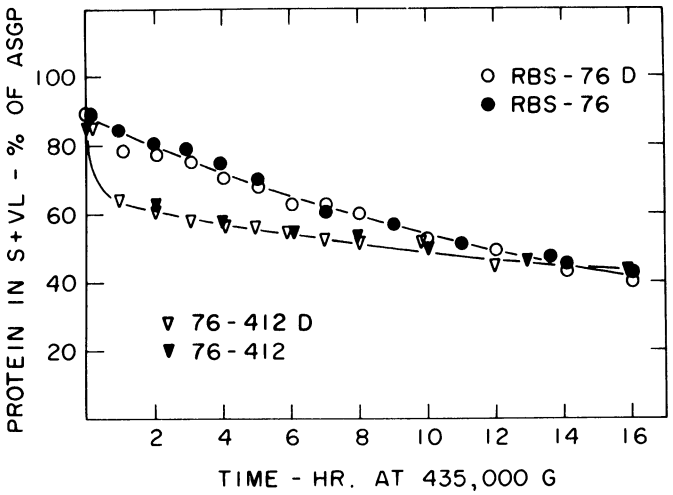

Fig. 9. Effect of removing the total free lipids from flours on the rates of sedimentation of their acid-solubilized gluten proteins at 435,000 × *g*. All fractions were centrifuged at 100,000 × *g* to remove their pellet proteins before the 435,000 ×*g* centrifugation. Plot shows the amount of protein in the combined supernatant (S) and viscous layer (VL) fractions after centrifugation for periods of 1–16 hr. RBS-76 D and 76-412 D flours were identical to RBS-76 and 76-412 flours, except the former were defatted with petroleum ether before their glutens were washed out.

complexes (Hoseney et al 1971), the sedimentation rates of the pellet and some of the gel glutenins apparently increased. Removing the total free lipids from the good-quality (RBS-76) flour before washing out its gluten did not materially affect the sedimentation properties of its ASGP. We conclude that the interactions between gliadin and pellet glutenin of the poor-quality flour are weaker than those of the relatively weak gliadin-lipid-glutenin complex. In the good-quality variety, the interactions between gliadin and pellet glutenin may be as strong as those between the proteins and lipids of nondefatted flour. We further suggest that the tenacity with which glutenin proteins interact with both gliadin proteins and free lipids may be the physicochemical criterion for why some wheat varieties have poor and others good loaf volume potentials.

Goforth et al (1977), in their studies on the effect of strength and concentration of acid on the functional properties of solubilized glutens, suggested that the protein-protein and/or protein-lipid interactions of poor-quality bread flours may be weaker than those of good-quality flours.

Removing the free lipids from a flour before washing out its gluten did not appreciably affect sedimentation properties (at 435,000 × *g*) of the 100,000 × *g* supernatant of either the poor- or the good-quality flour (Fig. 9). At the beginning of the 435,000 × *g* centrifugation, the supernatant contained the gel, VL, and supernatant proteins. Because the amount of protein in the combined supernatant plus VL fraction was not appreciably affected by defatting, the amount of protein in the gel fraction could not have been affected by defatting.

Specifications of Protein Fractions for Reconstitution

The high molecular weight proteins, presumably glutenin, sedimented at 100,000 × *g* as a dense, light brown, rubbery, relatively insoluble pellet. The poor-quality 76-412 pellet material sedimented in 35 min, and that of the good-quality RBS-76 in 2 hr. The (presumably) low molecular weight glutenin proteins sedimented at 435,000 × *g* as an off-white, relatively soluble, mesomorphic layer or gel. The 76-412 gel sedimented in 6 hr, and the RBS-76 gel in 10 hr. The high molecular weight gliadin proteins also sedimented at 435,000 × *g* to form a clear, colorless, viscous layer. The 76-412 VL formed in 6 hr, and that of RBS-76 in about 10 hr. The low molecular weight gliadins of both flours remained in the supernatants. For those conditions, the ratio of the ASGP in the combined pellet and gel fractions to that in the combined viscous layer and neutralized supernatant fractions was approximately 48:52 for both good- and poor-quality flours. The functional properties (loaf volume and dough mixing) of the gel, VL, and supernatant fractions of both good- and poor-quality flours have been reported (Finney et al 1982).

ACKNOWLEDGMENT

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