

COMMUNICATION TO THE EDITOR
Separation of Glutenin from Gliadin by Ultracentrifugation¹

Cereal Chemistry 53(4): 608-612

TO THE EDITOR:

Described is an ultracentrifugation procedure for physically separating glutenin from gliadin after solubilizing gluten in 0.005*N* lactic acid.

Osborne (1) in 1907 originally separated gliadin from glutenin using solubility as a criterion. But use of alcohol according to his technique renders those proteins nonfunctional (2) in breadmaking. Gel filtration chromatography (3,4) and fractional precipitation (5,6,7) have also been used for the separation of those two protein fractions. An ideal separating scheme would be one that requires minimum fractionating time, yields relatively pure fractions, and retains the functional properties of the proteins.

Figure 1 is a flow diagram for the separation of glutenin from gliadin by a Beckman Model L2-65B preparative ultracentrifuge. Hosney *et al.* (8) separated

¹This work constitutes part of the dissertation submitted by D. R. Goforth to the Graduate Faculty of Kansas State University in partial fulfillment of the requirements for the Ph.D. degree.

Mention of specific instruments or trade names is made for identification purposes only and does not imply any endorsement by the U.S. Government.

about 32% of the glutenin from solubilized gluten by ultracentrifugation at $100,000 \times g$ for 5 hr. We found that, by increasing the relative centrifugal force to $435,000 \times g$ and extending the time to 12 hr, virtually all of the glutenin can be removed from acid-soluble gluten (Fig. 2). When the acid-soluble gluten was fractionated into the classical gliadin and glutenin fractions with 70% ethanol, the two fractions were present in about equal quantities, which data served as the

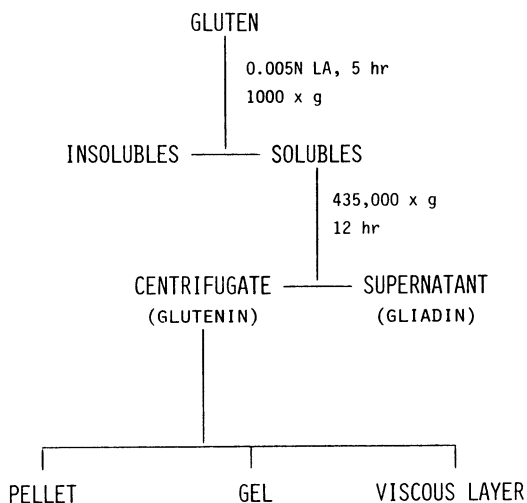


Fig. 1. Scheme for separating glutenin from gliadin proteins by ultracentrifugation.

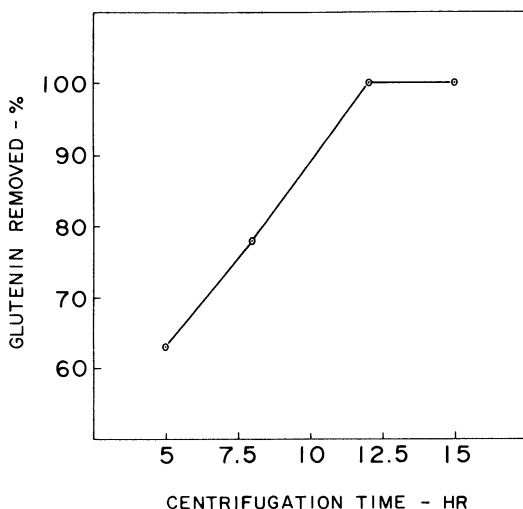


Fig. 2. Effect of ultracentrifugation time at $435,000 \times g$ on removal of glutenin from gliadin proteins of RBS gluten solubilized in 0.005N lactic acid.

basis for the amount of glutenin removed by ultracentrifugation. Enough protein was sedimented to account for about 50% of the total protein, or 100% of the glutenin (Fig. 2). Protein was measured by the Kjeldahl method ($N \times 5.7$).

Two fractions from the ultracentrifugation were analyzed by gel electrophoresis (7), with sample sizes adjusted so that each contained 2.5 mg protein (Fig. 3). RBS and 401 flours had medium and long mixing requirements, respectively, but both had good loaf-volume potential; 405 flour had a short mixing requirement and a poor loaf-volume potential. Patterns 1, 2, and 3 are for RBS, 401, and 405 supernatants, respectively, obtained by ultracentrifugation at $435,000 \times g$ for 5 hr (435-5S). Gliadin proteins are the slow major bands that migrated into the gel, and the faster bands are albumins and globulins. The high-molecular-weight glutenin remained at the origin. The material that migrated

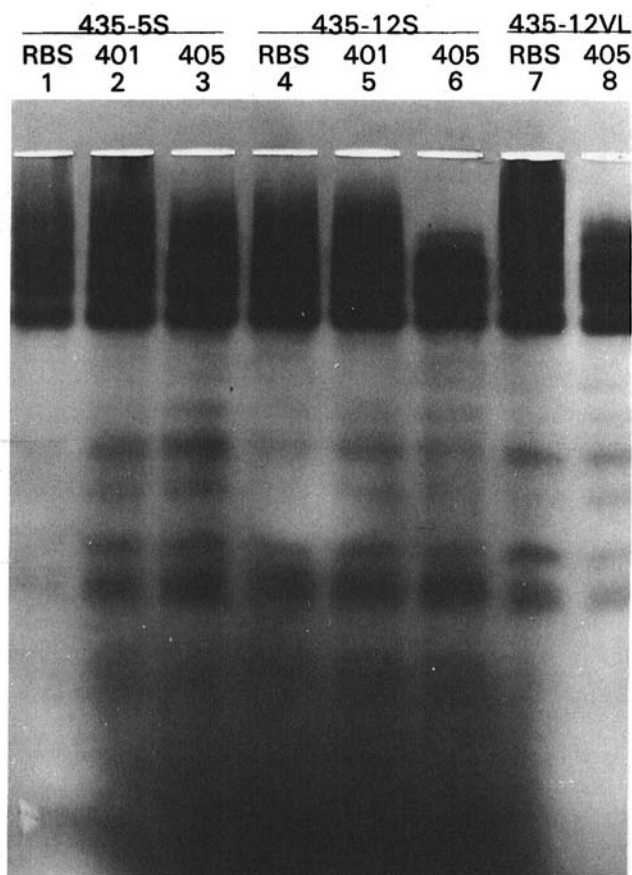


Fig. 3. Starch-gel electrophoretic patterns of lactic acid-solubilized gluten fractions obtained by ultracentrifugation at $435,000 \times g$ (435) for 5 and 12 hr. S and VL are abbreviations for supernatant and viscous layer, respectively. RBS and 401 represent good-quality flours, and 405, a poor-quality flour.

poorly or streaked into the gel is considered to be low-molecular-weight glutenin or high-molecular-weight gliadin. Streaking and material retained at the origins were evident for 1 and 2 (RBS and 401); there was relatively no streaking and no material remaining at the origin for sample 3 (405). The electrophoretic patterns of the supernatants of RBS and 401 after 12 hr of centrifugation (435-12S) showed no material at the origin and relatively little streaking (patterns 4 and 5). The corresponding pattern for 405 showed no streaking (pattern 6).

Visual examination of the centrifugates showed that the 12-hr samples contained a viscous layer not readily apparent in the 5-hr samples. The centrifugate, consisting of three parts, was made up of a small, rubbery, striated pellet that was surrounded by a mesomorphic or gel layer that adhered to a part of the bottom and side of the centrifuge tube (corresponding to angle of tube). The gel layer with its embedded pellet was covered by a clear viscous layer that occupied the remainder of the bottom half-inch of the centrifuge tube. Starch gel patterns are not shown for the gel or pellet fractions. The pellet consisted mainly of high-molecular-weight glutenin (insoluble glutenin) not soluble in hydrogen-bond breaking agents (8M urea), but dispersible in disulfide-bond breaking agents (β -mercaptoethanol). The gel fraction, on the other hand, was soluble in 8M urea and considered to be low-molecular-weight glutenin (soluble glutenin). Starch-gel pattern 7 of the viscous layer of RBS obtained by centrifuging at $435,000 \times g$ for 12 hr (435-12VL) clearly showed that some of the high-molecular-weight, low-mobility, and streaking proteins evident in the 5-hr supernatant of RBS (pattern 1) had settled in the viscous layer of the 12-hr centrifugate. Streaking in pattern 7 was more dense than in pattern 1 because the viscous-layer proteins applied at the origin of the gel of RBS were about 3.5 times as concentrated as they occurred in the 435-5S fraction. The 435-12VL pattern of 401 (not shown in figure) was the same as that of RBS 435-12VL. The streaking proteins of 405 (pattern 8) were not as concentrated as those in pattern 7 (RBS), because centrifugation for 12 instead of 5 hr caused about 40% more protein to be in the pellet and gel of 405, and thus there was less in the viscous layer.

The gliadin proteins observed in the viscous layer of RBS (pattern 7) may have been present because of an association with low-molecular-weight glutenin proteins. As noted above, the viscous layer of 405 contained little protein that streaked (pattern 8), but contained proteins that had mobilities similar to those of the gliadin proteins. Centrifugation for 12 instead of 5 hr put about 40% more protein in the gel and pellet of 405 than in those of RBS or 401. Starch gel patterns indicated that the protein in the viscous layer of 405 was gliadin protein. In general, patterns 3, 6, and 8 suggest that the protein components present in those fractions of the poor variety, 405, were less complex than those of the good varieties, RBS and 401. Two explanations for those data are: 1) the glutenin of the poor-quality flour is higher in molecular weight than that of the good-quality flours, so that it sediments faster, and/or 2) the 405 glutenin protein has a greater tendency to aggregate with itself than that of RBS or 401, causing it to sediment faster. A possible explanation for gliadin-like proteins being unexpectedly sedimented, particularly in the viscous layer of 405, is that they self-associated under the conditions of centrifugation. When that fraction was solubilized and

subjected to electrophoresis in a dissociating medium (3M urea), however, the proteins dissociated to their original state. The gliadin proteins of RBS and 401 also sediment if given enough centrifugation time (20 hr) at $435,000 \times g$.

Physically separating glutenin from gliadin proteins of wheat flour by ultracentrifugation may have profound ramifications in studies that relate those proteins to functional (breadmaking) properties.

D. R. GOFORTH

K. F. FINNEY

U.S. Grain Marketing Research Center
Agricultural Research Service
U.S. Department of Agriculture
Manhattan, KS 66502

Acknowledgment

The authors wish to acknowledge R. C. Hosenev for helpful discussions related to this work.

Literature Cited

1. OSBORNE, T. B. The proteins of the wheat kernel. Carnegie Inst.: Washington, D.C. (1907).
2. HOSENEY, R. C., FINNEY, K. F., POMERANZ, Y., and SHOGREN, M. D. Functional (breadmaking) and biochemical properties of wheat flour components. IV. Gluten protein fractionation by solubilizing in 70% ethyl alcohol and in dilute lactic acid. *Cereal Chem.* 46: 495 (1969).
3. JONES, R. W., BABCOCK, G. E., TAYLOR, N. W., and DIMLER, R. J. Fractionation of wheat gluten by gel filtration. *Cereal Chem.* 40: 409 (1963).
4. CROW, M. J. A., and ROTHFUS, J. A. Chromatography of proteins from wheat gluten on polyacrylamide gel. *Cereal Chem.* 45: 413 (1968).
5. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. *Arch. Biochem. Biophys.* 84: 363 (1959).
6. EWART, J. A. D. Fractional extraction of cereal flour proteins. *J. Sci. Food Agr.* 19: 241 (1968).
7. SHOGREN, M. D., FINNEY, K. F., and HOSENEY, R. C. Functional (breadmaking) and biochemical properties of wheat flour components. I. Solubilizing gluten and flour protein. *Cereal Chem.* 46: 93 (1969).
8. HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. *Cereal Chem.* 46: 126 (1969).