Gliadin Proteins from Different Varieties of Wheats[1]

F. R. HUEBNER and **J. A. ROTHFUS,** Northern Regional Research Laboratory, Peoria, Illinois

ABSTRACT

Protein compositions of gliadin fractions from several varieties of common, durum, and club wheats were compared by column chromatography on sulfoethyl cellulose and by starch-gel electrophoresis. Compositional differences among the wheats studied were greatest between varieties representing different classes and least between varieties of the same class. Two hard red winter wheats, Red Chief, a poor-baking-quality wheat, and Comanche, a good-quality wheat, showed only a few significant differences. All varieties analyzed contained proteins analogous to the γ_1-gliadin of Comanche. In addition, several other components from different classes of wheat had nearly identical chromatographic and electrophoretic properties. Compared to gliadin from Comanche *(Triticum aestivum,* ssp. *vulgare),* the gliadin of Omar, a club wheat *(T. aestivum,* ssp. *compactum),* contained a larger number of β-gliadins and fewer α-gliadins, whereas gliadin from Lakota, a durum wheat *(T. durum),* differed primarily by having at least three additional gamma-gliadinlike components. Differences among varieties in the quantitative distribution of proteins were also noted. Amino acid analysis of isolated components from different varieties showed that γ_1-gliadins have nearly identical amino acid composition. A γ-gliadin from Lakota and γ_3-gliadin from Red Chief also have nearly the same amino acid content.

The quality of baked bread may be influenced by a number of variables, including the presence of additives, method of mixing, and other physical treatment of the dough, but the extent to which any of these factors can improve bread is limited by the composition of flour and the molecular structure of the flour components. Although the starch (1), enzymes (2), and lipids (3) in a flour affect its baking properties, protein, especially gluten, appears to be the constituent uniquely important in the production of the expanded dough matrix in baked goods (4–6).

Quantitative effects of protein in flour are evidenced by the well-known differences in baking qualities of flours from different types of wheat. Whereas bread is produced primarily from hard wheats, the soft wheats, lower in protein, are chiefly consumed as cake, pastry, and crackers. Since durum wheats, which generally contain the most protein, yield gluten that is too cohesive for general baking, they are used to make pasta products such as macaroni and spaghetti (7).

Qualitative differences in flour protein are less obvious, even though it is well known that flours from similar varieties of wheat with nearly the same total protein content differ significantly in baking characteristics (4,5,8). Lee and Wrigley (9) showed that gluten proteins from different varieties of Australian wheat gave similar, yet characteristic, patterns upon chromatography and zone electrophoresis. Unfortunately, limitations in the methods of resolution available at the time prevented correlation of specific protein compositions with baking qualities of the wheats studied.

[1]Contribution from the Northern Regional Research Laboratory, Peoria, Illinois 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Presented, in part, at the 52nd Annual Meeting, Los Angeles, California, April 1967. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Since then, Huebner and Wall (10) developed an improved chromatographic fractionation of gliadin proteins on sulfoethyl cellulose (SEC). When applied to gliadin from a hard red winter (HRW) wheat, Ponca, this method of separation resolved the γ-gliadin fraction into three electrophoretically distinct proteins, differing in amino acid composition (11). This report compares chromatography on SEC and subsequent electrophoresis of proteins in the gliadin fractions from ten wheat varieties representing five different classes commonly grown in the United States. Amino acid analyses on selected proteins from these different varieties are also compared.

MATERIALS AND METHODS

Wheat samples were taken from Laboratory stock. The two HRW wheats were Red Chief 1959, from Manhattan, Kans., milled in 1960 and kept at –17°C. until used, and Comanche 1963, from Kinsley, Kans. Two hard red spring (HRS) wheats were Lee 1962, from Culbertson, Mont., and Selkirk 1962, from Redstone, Mont. Two soft red winter (SRW) wheats were Knox 1962, from Huntington, Ind., and Seneca 1963, from Sandusky, Ohio. The soft white winter (SWW) and white club (WC) wheats were, respectively, Brevor and Omar 1963, from Pullman, Wash. The durum wheats were Lakota and Wells 1963, from Carrington, N. Dak.

Other than Red Chief, the wheats were milled before use in a Buhler mill with 59 to 73% extraction on the different wheats. Gliadin was prepared by 0.1*N* acetic acid extraction of the gluten ball and fractionation in neutral 70% ethanol according to the method of Jones *et al.* (12). Since the gluten ball from the soft and durum wheat flours was too weak to allow good recoveries by washing out the starch with 0.1% NaCl, the gluten was extracted directly from the flour with 0.1*N* acetic acid in a Waring Blendor, and then the starch was removed by centrifugation. High-molecular-weight components in the gliadin were eliminated by gel filtration on Sephadex G-100 (Pharmacia, Inc., Uppsala, Sweden) as described by Beckwith *et al.* (13). SEC (Bio-Rad Laboratories, Richmond, Calif., 0.2 meq./g.) in the sodium form was suspended in water and decanted three or four times to remove fines. A column 3.9 × 50 cm. packed with this purified resin was equilibrated with buffer just before application of the sample.

N,N-Dimethylformamide (DMF) (Matheson Co., East Rutherford, N.J.) was used without further purification.

Ion-Exchange Chromatography

Chromatography on SEC was carried out as previously described (11) in 2*M* DMF, 0.03*N* acetic acid, and 0.015*N* HCl at pH 2.2. The buffer was prepared immediately before use. A nonlinear gradient of NaCl from 0.03*M* to 0.16*M* was generated in an automatic chromatography apparatus (Beckman Model 130 Spectrochrom) according to the gradient cam duplicated in Fig. 1. Tubes under each peak were combined and dialyzed exhaustively against 0.01*N* acetic acid before lyophilization.

Gel Filtration

Before amino acid analysis, protein fractions from the SEC column were further purified by gel filtration (11) on either a 2.3 × 176-cm. or a

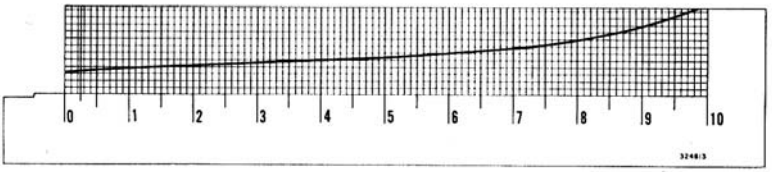

Fig. 1. Template of program cam used to generate the salt gradient in the chromatography of gliadin on sulfoethyl cellulose (SEC). Program cam was cut along the solid curved line. Abscissa represents gradient volume, 0 to 100%; ordinate, salt concentration, 0 to 0.16*M*.

4.2 × 240-cm. column of Sephadex G-50 fine beads in 0.05*N* acetic acid at room temperature with a flow rate of 2.5–3.0 ml./hr./cm.^{2} Samples of less than 50 mg. were applied to the smaller column and those up to 350 mg., to the larger. Column effluents were passed through an automatic recording spectrophotometer (Vanguard) set at 280 mμ, ahead of a fraction collector. The desired tubes were combined and lyophilized directly. On a weight basis, the recovery was 95 to 100%.

Starch-Gel Electrophoresis

Starch-gel electrophoresis was carried out in 3*M* urea, 0.005*M* aluminum lactate, and 0.02*M* lactic acid buffer as described by Woychik *et al.* (14) and modified by Beckwith *et al.* (13). The buffer was kept at 4°C. and discarded if unused after approximately 1 week. Aliquants (about 25 μl.) of 1 to 2% solutions of protein in the electrophoresis buffer were absorbed on small squares of filter paper, inserted into the gel, and then electrophoresed for 3.5 to 4 hr. at about 110 v. with cooling.

Amino Acid Analysis

Four samples (about 3 mg. each) of protein were placed in separate 2-ml. hydrolysis ampules, dissolved in 2 ml. of twice-distilled, constant-boiling HCl, and frozen in solid carbon dioxide-acetone. The ampules were then evacuated and the contents allowed to melt under vacuum to remove dissolved air. The ampules were then flushed with nitrogen re-evacuated, and sealed. Duplicate samples were heated at 110°C. ± 1°C. for 24 and 48 hr., respectively, after which they were evaporated to dryness, dissolved in water, evaporated twice more, and finally diluted with pH 2.2 citrate buffer. Analyses were carried out by the method of Benson and Patterson (15) on an automatic amino acid analyzer (Phoenix Model K-8000) modified for accelerated operation. The apparatus was further modified by addition of an Infotronics (Houston, Texas) automatic integrating system, which was used in conjunction with an IBM 1130 computer for automatic computation of analysis results (16). Nitrogen content of gliadin fractions was determined by micro-Kjeldahl analyses.

EXPERIMENTAL AND RESULTS

Satisfactory correlation of the properties and compositions of protein fractions from wheat flour has been limited by the resolving power of the techniques used to fractionate the flour. Recently Huebner and Wall (10) developed an improved method of chromatography that facilitated the iso-

lation and partial characterization of three chemically distinct proteins from the γ-gliadin fraction of HRW wheat, Ponca (11). A combination of this improved method of column chromatography and gel electrophoresis seemed well suited for application to the comparative analysis of gliadin from different varieties of wheat. Throughout this paper, areas of the gel-electrophoretic patterns have been labeled α, β, and γ in keeping with the nomenclature suggested by Woychik *et al.* (14). In gels where these regions are not well defined, the designations are approximate and are useful only in terms of the discussion following.

The wheats used in this study were selected to represent different classes and, where possible, different gluten qualities and baking characteristics (8) within the same class. Yields of gluten from the different varieties ranged from 9 to 11% (dry weight) for hard wheats, 6.5 to 8% for soft wheats, and 11.5 to 13% for the durum wheats. The glutens, in turn, generally consisted of 60 to 70% gliadin and 30 to 40% glutenin. Before analysis, glutenins and ω-gliadins were excluded from the gliadin prepared from each of the wheats by precipitation from 70% ethanol and then chromatography on Sephadex G-100 (13). Although the ω-gliadin fraction is small, it contains components that might cause problems in fractionation if not removed. Chromatography on SEC was then carried out with the same amount of gliadin from each variety studied.

Chromatographic fractionation of the gliadin from all the wheats was standardized with automatic equipment. Figure 1 shows the template of the program cam used to generate the salt gradient in the Beckman Spectrochrom. This figure can also be used to calculate the concentrations required in a nine-chamber variable gradient device. Exact reproduction of the gradient was necessary to duplicate resolution of the proteins on the SEC column. The required salt gradient could not be obtained readily by analysis of the column effluent, owing to changes that occurred during chromatography.

Even though automation reduced the number of variables affecting generation of a continuous gradient, other opportunities for variation in chromatography still exist. For example, in our present studies, the α-gliadin components were resolved somewhat better than in previous work with Ponca, a HRW wheat (11). Presumably this improved resolution resulted from subtle changes in column packing, since the same gradient was used in both investigations. Because slight variations between chromatographic runs were evident, we have considered relative chromatographic and electrophoretic mobilities as important as exact elution volume in comparing the gliadin proteins from different wheats.

HRW Wheats

Figure 2 shows chromatographic fractionation of gliadins from two HRW wheats—Red Chief, a poor-baking wheat, and Comanche, a good-baking wheat. Fractions are numbered starting at an effluent volume of 1 liter. The numbers only identify fractions from individual varieties and

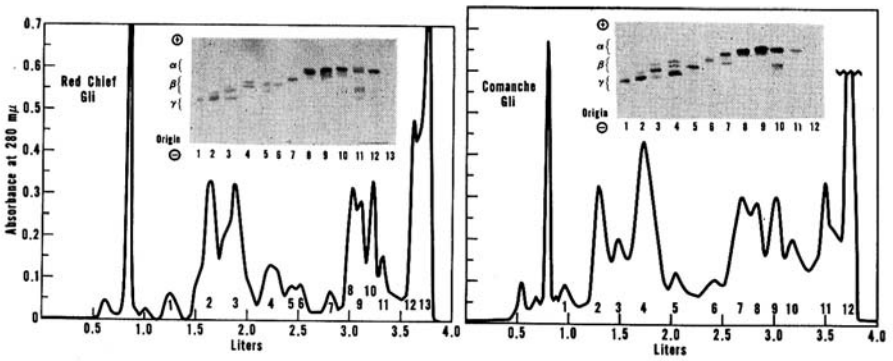

Fig. 2. Chromatographic separation on SEC of gliadin from Red Chief and Comanche HRW wheats and starch-gel-electrophoretic patterns of gliadin fractions.

are not intended to imply identity to similarly numbered fractions from other varieties.

The unnumbered peaks between 0.5 and 1.0 liter consisted of very small amounts of proteinlike substances which absorbed light more strongly at 260 than at 280 mμ. In addition, material in the tall slender peak was slightly yellow. These peaks were all eluted ahead of the salt gradient. The large peak which generally was present at the end of the elution pattern consisted of water-soluble components eluted together because of a stepwise increase in salt concentration to about 0.25*M*. These materials were usually included in the starch-gel electrophoresis but did not show up well, because of either their greater mobility or decreased affinity for the nigrosine dye.

The elution and electrophoresis patterns vary the most in fractions 3 through 8. Red Chief may have an extra component in fractions 3 to 5, and Comanche fraction 7 may contain a different protein. Otherwise, these two gliadins give essentially identical patterns. The gliadin pattern from Ponca (11), another good-baking-quality HRW wheat, also resembles that of Red Chief very closely, but Red Chief and Ponca may differ by at least one component.

HRS Wheats

Elution and gel patterns of gliadin from two HRS wheats, Lee and Selkirk, are shown in Fig. 3. Both these wheats yield good-quality gluten; however, Selkirk is noted for higher loaf volume in relation to its total protein content.

There are more differences in the two HRS wheats than were apparent in the two HRW wheats. Nearly every fraction in Lee has a chromatographic counterpart in Selkirk. The first three fractions from each of the HRS wheats are quite similar electrophoretically. The elution volume of fraction 4 from Lee corresponds to fractions 4, 5, and 6 from Selkirk, but electrophoretically fraction 6 of Selkirk appears to contain a different protein. Similarly, fraction 8 from Selkirk contains a component that is not present in fraction 6 from Lee. Fraction 7 from Lee and fraction 10 from Selkirk emerge at the same volume, but the proteins contained in these

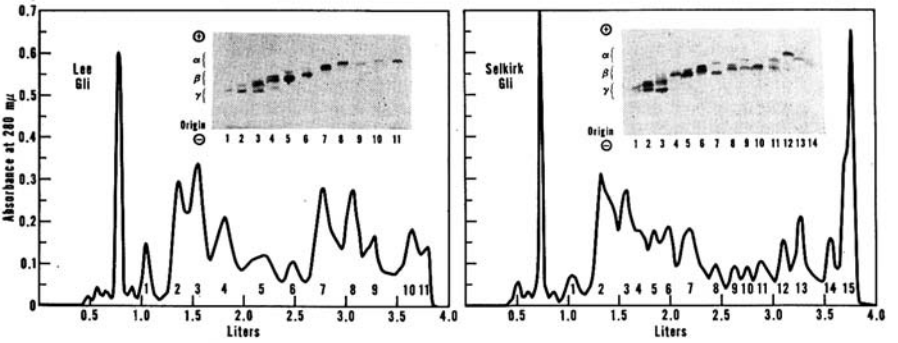

Fig. 3. Chromatographic separation on SEC of gliadin from Lee and Selkirk HRS wheats and starch-gel-electrophoretic patterns of gliadin fractions.

fractions differ both in quantity and electrophoretic mobility. Quantitative differences are also apparent between fractions 8 of Lee and 12 of Selkirk, but the proteins have nearly identical chromatographic and electrophoretic mobilities. In general, Lee closely resembles the HRW wheats. Selkirk, however, appears to contain more beta-components and fewer alpha-components than are present in either Lee or the HRW wheats.

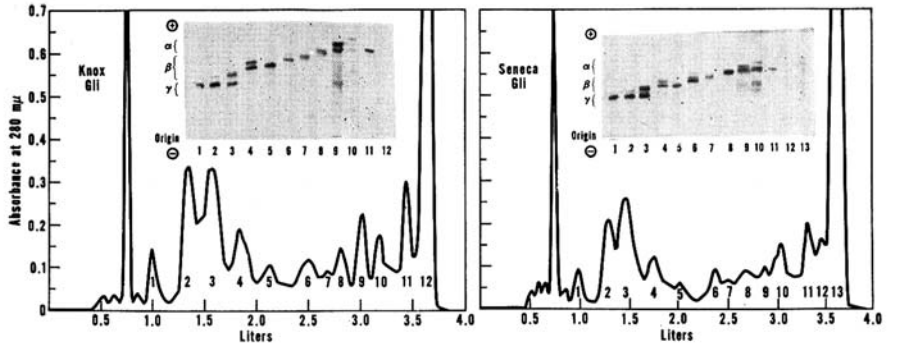

Fig. 4. Chromatographic separation on SEC of gliadin from Knox and Seneca SRW wheats and starch-gel-electrophoretic patterns of gliadin fractions.

SRW Wheats

Figure 4 shows the gel electrophoresis and elution patterns of gliadin from two SRW wheats, Knox and Seneca. Gluten from each of these wheats is rated good quality for soft wheats. Knox is sometimes rated excellent.

The electrophoretic patterns of these two varieties are quite similar. The additional bands in Seneca fractions 6 and 9 and in Knox fraction 7 may be real differences, but they could also be artifacts of incomplete resolution or differences in protein concentration. In comparison with gliadins from other classes of wheat, the patterns of these varieties are similar to those from HRW wheats, but there appears to be one or two more β-gliadins and one less α-gliadin in Knox and Seneca. In addition, two of the α-gliadins in fractions 9 and 10 of either soft wheat seemed to migrate further during electrophoresis.

Durum Wheats

Chromatographic separations of gliadins from Lakota and Wells, two durum wheats, are given in Fig. 5. Lakota yields stronger gluten and has

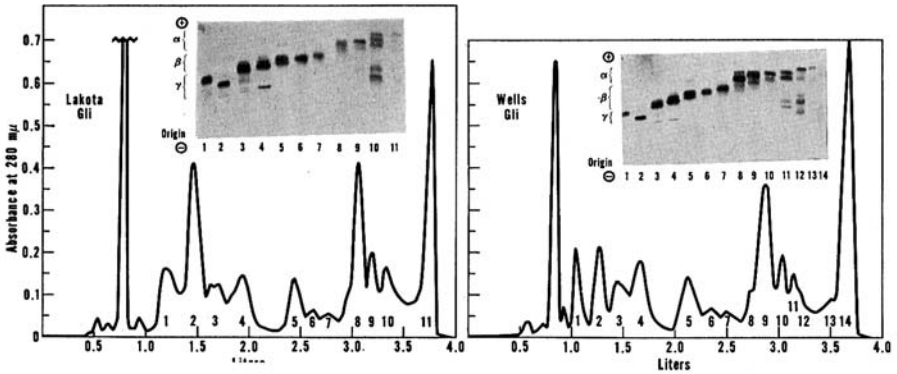

Fig. 5. Chromatographic separation on SEC of gliadin from Lakota and Wells durum wheats and starch-gel-electrophoretic patterns of gliadin fractions.

better macaroni cooking qualities than Wells.

Even though the elution pattern for Lakota is displaced slightly, the chromatographic and electrophoretic distributions of the proteins in these two durum wheats are nearly identical from a qualitative standpoint. Slight differences in the alpha-region of the electrophoretic patterns may be due more to the way samples were taken than to actual differences between the two gliadins. There is a significant difference between the durum wheat gliadins and gliadins from other varieties, however. The durums appear to contain little or none of the γ_2- or γ_3-gliadins found in Ponca (11) and, instead, contain five or six new components with slower electrophoretic mobilities than the Ponca γ-gliadins. There may also be as many as eight α-gliadins in the durum varieties, significantly more than were found in the soft wheats.

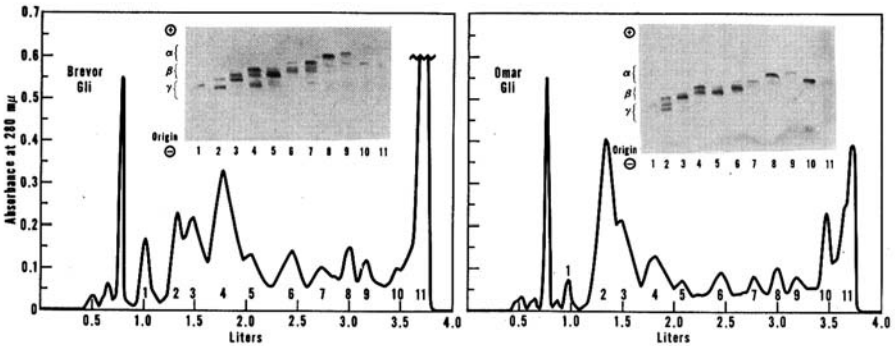

Fig. 6. Chromatographic separation on SEC of gliadin from Brevor and Omar SWW and club wheats and starch-gel-electrophoretic patterns of gliadin fractions.

SWW and WC Wheats

Figure 6 shows the chromatographic and electrophoretic patterns of gliadins from Brevor, a SWW wheat, and Omar, a WC wheat. Brevor is only satisfactory for general-purpose flour and cake flour, but for a soft wheat, Omar has excellent milling and baking properties.

While electrophoretic patterns for these two varieties look similar, there are a few specific differences. A different component of low electrophoretic mobility is apparent in fraction 2 of Omar. The γ_3-gliadin common in all but the durums also seems to be displaced or absent from Brevor and may be present in only trace amounts in Omar.

Weight Distribution of Gliadin Components

Quantitative variations in the various wheats are also apparent from the elution patterns. Whereas HRW and durum wheats have several optically dense alpha-components, soft and club wheats contain comparatively low levels of similar constituents. Surprisingly, the same difference is also apparent in the alpha-regions of the two HRS wheats, Lee and Selkirk. The SWW wheat, Brevor, and the WC wheat, Omar, differ most in fractions 2 and 4. The greatest quantitative differences in 280 mμ absorbance between chromatographically and electrophoretically similar fractions from varieties in the same class are apparent in fraction 8 of Lee and fraction 12 of Selkirk, fractions 2 and 9 in the SRW wheats, and fraction 2 of the durum wheats.

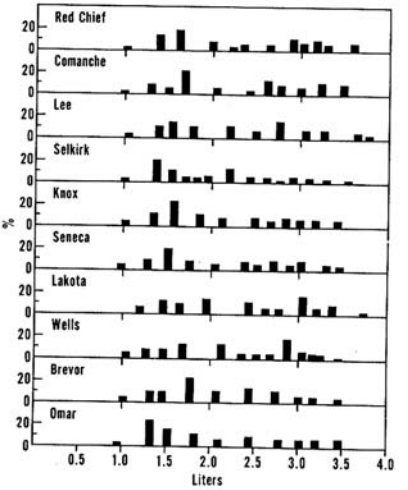

Fig. 7. Distribution of gliadin proteins from different wheats on sulfoethyl cellulose. Vertical bars represent percent of total dry weight recovered.

Figure 7 shows the relative distribution of the gliadins recovered from each variety of wheat on a dry-weight basis. Generally, the first five fractions account for approximately 50% or more of the total recovered from each variety. In hard and durum wheats at least one of the subsequent fractions contains a comparatively high proportion of the remaining weight. In contrast, this sort of distribution does not occur in the club wheat or in

the soft wheats, Lee and Knox. Brevor, however, has an intermediate distribution. The distributions of gliadin proteins on a dry-weight basis correlate well with the elution patterns, in that fractions with high absorbance correspond to more protein. One notable exception is fraction 2 of Lakota, which absorbed ultraviolet light much more strongly than adjacent fractions or the corresponding fraction in Wells, yet accounted for only 12% of the material recovered. Presumably this difference reflects a fundamental difference in the composition of a constituent in fraction 2 from Lakota.

Chemical Comparison of Gliadin Components

Fraction 1 from each variety of gliadin behaved as a single component chromatographically and electrophoretically and appeared similar to the γ_1-gliadin of Ponca (11). This fraction from four varieties was purified further and analyzed to see how nearly alike they were in amino acid composition. Fraction 1 from each variety chosen for amino acid analysis was first chromatographed on G-50 (fine) Sephadex as described under "Materials and Methods." This step eliminated small amounts of impurities and gave material that migrated as a single component upon electrophoresis in starch gel. The major component in fraction 2 from Lakota and γ_3-gliadin from Red Chief were purified similarly. Results of the analyses of these gliadins are listed in Table I. The amino acid analyses in terms of g. of amino acid

TABLE I

AMINO ACID ANALYSES OF GLIADIN COMPONENTS FROM DIFFERENT VARIETIES OF WHEAT[a]

	AMINO ACID (g./16 g. N)[b]						NEAREST EVEN INTEGER RESIDUES PER MINIMAL MOLECULAR WEIGHT[b]					
	γ_1				γ_3		γ_1				γ_3	
AMINO ACID	Red Chief	Knox	Wells	Brevor	Red Chief	No. 2 Lakota	Red Chief	Knox	Wells	Brevor	Red Chief	No. 2 Lakota
Lys	trace	trace	trace	trace	0.7	0.7	0	0	0	0	1	1
His	1.6	1.6	1.5	1.4	1.5	1.6	2	2	2	2	2	2
Arg	1.8	1.8	1.8	1.6	1.5	1.2	2	2	2	2	2	1
Asp	2.9	2.8	2.9	2.6	1.9	1.8	4	4	4	4	3	3
Thr	1.7	1.6	1.6	1.6	2.2	2.2	3	3	3	3	4	4
Ser	4.3	3.9	3.8	4.1	3.7	3.7	8	7	7	8	7	7
Glu	45.8	43.3	42.7	39.2	40.1	42.0	56	57	56	57	54	54
Pro	14.5	13.6	13.6	12.4	16.8	17.3	23	23	23	23	29	29
Gly	1.4	1.3	1.3	1.2	1.4	1.7	3	3	3	3	4	4
Ala	2.3	2.2	2.3	2.0	2.0	2.2	5	5	5	5	5	5
Val	3.8	3.7	3.7	3.6	3.2	3.5	6	6	7	7	6	6
Met	0.9	0.7	0.8	0.6	1.2	1.1	1	1	1	1	2	2
Ileu	4.4	4.4	4.4	4.0	3.8	4.3	6	7	7	7	6	6
Leu	7.0	6.6	6.7	6.2	5.8	6.2	10	10	10	10	9	9
Tyr	3.5	2.7	3.0	2.2	0.4	0.4	3	3	3	3	1	1
Phe	5.2	4.4	4.4	4.1	6.6	7.3	5	5	5	5	8	8
NH_3	4.5	4.3	3.8	3.7	4.1	4.2	48	48	45	47	47	47

[a]Values represent averages of duplicate analyses of samples hydrolyzed for 24 and 48 hr. *in vacuo* at 110° in 6*N* HCl. Lack of samples prevented satisfactory analyses of tryptophan and cystine.

[b]See "Experimental and Results" section for explanation.

per 16 g. nitrogen (Table I) were calculated on the basis of nitrogen content in aliquots of each hydrolyzed sample. On the basis of this same nitrogen value, the nitrogen content of each dry protein before hydrolysis was 17.1 ± 0.4%, and nitrogen recoveries after analysis were 80 to 90%. Sim-

ilarly, the weight of amino acids recovered in each analysis was only 86 to 92% of the weight of sample hydrolyzed. Values slightly less than 100% might have been expected, since tryptophan was not determined, but it is more likely that low values and minor differences between analyses were due to small losses of material while the microsize samples were being carried through the steps involved in hydrolysis and amino acid analysis. Accordingly, if the weight of amino acids recovered is used as a measure of the amount of protein actually analyzed, nitrogen recoveries were 94 to 98%. Minimum molecular residue ratios (Table I) were calculated on the basis of one residue of methionine in the γ_1-gliadins and one residue of lysine in the γ_3-gliadin from Red Chief and γ-gliadin of Lakota. Minimal molecular weights from these data would be 16,000 to 17,000.

γ_1-Gliadins from Red Chief, Knox, Wells, and Brevor were essentially devoid of lysine. In this respect, as well as in terms of the remaining amino acids analyzed, these γ_1-gliadins were like the γ_1-gliadin from Ponca (11).

Greater variation was apparent when γ_3-gliadin from Red Chief was compared to γ_3-gliadin of Ponca (11). The protein from Red Chief, like that of Ponca, contained single residues of lysine and tyrosine, but eight less residues of glutamic acid and one residue less of valine, isoleucine, leucine, and phenylalanine.

The major protein from fraction 2 of Lakota (Fig. 5), which resembled γ_2-gliadin from Ponca (11) chromatographically but not electrophoretically, differed from Ponca by three residues of glutamic acid and single residues of arginine and eight other neutral amino acids. Surprisingly, this protein from Lakota was nearly identical in composition to the γ_3-gliadin from Red Chief.

DISCUSSION

Hydrated gluten has the combined properties characteristic of its components, glutenin and gliadin. Individually, neither of these materials is a satisfactory substitute for gluten in the baking process. In view of the importance of both components, interpretations of these studies on gliadin in terms of baking properties should await comparable data on glutenin. Nevertheless, the separation and characterization of gliadins from different varieties of wheat is an important preliminary to understanding the relation between the composition and properties of specific wheats.

Thus far, the patterns produced by chromatography and electrophoresis of the gliadin proteins from different varieties of wheat have been similar in general form, but no two have been identical. In general, varieties in different classes showed greater variation than varieties in the same class. Perhaps these results might have been anticipated from other knowledge of properties and baking characteristics, but it is indeed surprising that the patterns for Red Chief, a poor-baking-quality wheat, should be so similar to those of Ponca (11), a good-quality wheat in the same class. In the absence of knowledge of other factors responsible for the poor quality of Red Chief, it might be surmised that gliadin proteins, as a group, are of less import than glutenin in determining baking characteristics. Alterna-

tively, since Red Chief varied only slightly from other good-quality HRW wheats, differences in baking quality may depend more on the presence or absence of specific proteins—for example, enzymes or enzyme inhibitors. Further experimentation with the isolated proteins will be required to resolve this problem.

Similarly, the significance of quantitative differences in the chromatographic distribution of proteins from hard or durum and soft or club wheats (Fig. 7) remains to be elucidated, even though it is obvious that such variations could reflect fundamental differences in surface charge on the proteins in these classes of wheat.

In several wheats, most notably Lee and Selkirk (Fig. 3), some differences between varieties were due to the elution of proteins with similar electrophoretic mobilities in different chromatographic fractions. Such behavior could conceivably be caused by aggregation during chromatography, but this explanation seems doubtful in view of the chemical and electrophoretic differences Huebner *et al.* (11) found in purified γ-gliadins from Ponca.

Amino acid analyses of similar gliadin fractions from different classes of wheat (Table I) thus far have corroborated the analysis of Ponca (11) and have emphasized the effectiveness of combined chromatography and electrophoresis as a means of characterizing individual gliadin proteins. γ_1-Gliadins, which have similar chromatographic and electrophoretic properties, appear to have similar amino acid compositions. On the other hand, proteins that differ in electrophoretic or chromatographic mobility need not have completely different amino acid compositions, since variations in amino acid sequence or a single charged amino acid might be responsible for their different characteristics. This explanation may be true for the major component in fraction 2 from Lakota (Fig. 5), which resembles the γ_2-gliadin of Red Chief (Fig. 2) chromatographically, but which is more similar to the γ_3-gliadin of Red Chief in terms of amino acid composition.

Thus far, comparable γ-gliadin proteins from different varieties of wheat have shown no variation in lysine, histidine, or aspartic acid and have shown only minimal differences in glutamic acid and the remaining neutral amino acids. It remains to be seen whether proteins that are unique to a given variety or class of wheat will deviate significantly from the patterns established here.

Literature Cited

1. SPILLANE, P. A., and MCGOVERN, HELEN. Influence of environmental factors on the milling and baking behavior of some wheat varieties. Cereal Sci. Today 11: 441–443, 458 (1966).
2. POMERANZ, Y. Protein composition and breadmaking potentialities of wheat flour. Cereal Sci. Today 11: 192–194, 196 (1966).
3. POMERANZ, Y. A review of recent studies of wheat flour lipids in breadmaking. Baker's Dig. 40: 44–48 (1966).
4. FINNEY, K. F. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20: 381–396 (1943).
5. DIMLER, R. J. Gluten—the key to wheat's utility. Baker's Dig. 37: 52–57 (1963).
6. HOLME, J. A review of wheat flour proteins and their functional properties. Baker's Dig. 40: 38–42 (1966).

7. IRVINE, G. N. Durum wheat and paste products. *In* Wheat: Chemistry and Technology, I. Hlynka, ed.; chap. 12, p. 553. Monograph Series, Vol. III. American Association of Cereal Chemists: St. Paul, Minn. (1964).
8. BRIGGLE, L. W., and REITZ, L. P. Classification of Triticum species and of wheat varieties grown in the United States. Tech. Bull. 1278. U.S. Dept. Agr. (1963).
9. Lee, J. W., and Wrigley, C. W. The protein composition of gluten extracted from different wheats. Australian J. Exp. Agr. Animal Husbandry 3: 85–88 (1963).
10. HUEBNER, F. R., and WALL, J. S. Improved chromatographic separation of gliadin proteins on sulfoethyl cellulose. Cereal Chem. 43: 325–335 (1966).
11. HUEBNER, F. R., ROTHFUS, J. A., and WALL, J. S. Isolation and chemical comparison of different γ-gliadins from hard red winter wheat flour. Cereal Chem. 44: 221–229 (1967).
12. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84: 363–376 (1959).
13. BECKWITH, A. C., NIELSEN, H. C., WALL, J. S., and HUEBNER, F. R. Isolation and characterization of a high-molecular-weight protein from wheat gliadin. Cereal Chem. 43: 14–28 (1966).
14. WOYCHIK, J. H., BOUNDY, J. A., and DIMLER, R. J. Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Arch. Biochem. Biophys. 94: 477–482 (1961).
15. BENSON, J. V., JR., and PATTERSON, J. A. Accelerated automatic chromatographic analysis of amino acids on a spherical resin. Anal. Chem. 37: 1108–1110 (1965).
16. CAVINS, J. F., and FRIEDMAN, M. Automatic integration and computation of amino acid analyses. Cereal Chem. 45: 172–176 (1968).

[Received March 20, 1967. Accepted January 15, 1968]