Studies of Glutenin. II. Relation of Variety, Location of Growth, and Baking Quality to Molecular Weight Distribution of Subunits[1]

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ABSTRACT

Subunits obtained by reduction of glutenin from 26 hexaploid wheats of diverse baking quality were examined by SDS-polyacrylamide gel electrophoresis. The number of observable bands, which varied from ten to fourteen, was not characteristic of baking quality. Intervarietal differences in the number and molecular weight of the subunits cannot be used to characterize the baking potential among these hexaploid wheats. Genetically related varieties contained common glutenin subunits, the closeness of their electrophoretic patterns being dependent on their genetic similarity. Location of growth had no effect on the subunit composition of four varieties each grown at four locations.

Solubility fractionation of wheat-flour proteins from many varieties has led to the conclusion that the amounts of acetic acid-soluble and the insoluble residue protein (glutenin) are correlated with breadmaking quality (1,2). Because of their large particle sizes and general insolubility in aqueous systems, it has not been possible to determine their structure in the hope of relating it to the functional properties of dough.

A marked increase in the solubility of glutenin can be obtained by reductive cleavage of its disulfide bonds (3). Starch-gel electrophoresis (4) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5,6) of subunits obtained by reduction of glutenin from wheats of different varieties and classes indicated marked differences.

The present study, which was in progress at the time the work of Bietz and Wall (6) was reported, examines the SDS-PAGE patterns of the reduced glutenin of 26 bread wheats grown at four locations in western Canada. The aim of this work was to determine if the subunits of glutenin were related to baking quality and whether they were affected by environment, or simply genetically controlled.

MATERIALS AND METHODS

The varieties used, their parentage, loaf-volume per unit protein, and flour protein are the same as used in a previous study (1). These varieties are ideal for this study because of their diverse baking qualities and the genetic similarity of a number of the samples.

Molecular weight markers for the SDS-PAGE, and their source, are given in Table I.

Preparation of Glutenins

A modified Osborne fractionation scheme, as described by Chen and Bushuk (7), was used to prepare the glutenin proteins. Traces of gliadin and

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TABLE I. SDS-PAGE MOLECULAR WEIGHT MARKERS

Protein	Molecular Weight daltons	Source
γ-Globulins	160,000	Sigma
Bovine serum albumin - dimer	132,000	Calbiochem.
Bovine serum albumin - monomer	66,000	Calbiochem.
Ovalbumin	45,000	Sigma
Pepsin	35,000	Nutritional Biochem.
α-Chymotrypsin	21,600	Calbiochem.
Myoglobin	17,000	Calbiochem.
Trypsin inhibitor	14,300	Calbiochem.
Cytochrome C	12,400	Calbiochem.

low-molecular-weight proteins were removed from these preparations by dissolving 50 mg. of crude glutenin in 12.5 ml. of solvent comprising 0.1M acetic acid, 3M urea, and 0.01M hexadecyltrimethyl ammonium bromide (pH 3.5), stirring overnight with 25 mg. SE-Sephadex and centrifuging (8,9). The supernatant containing purified glutenin was dialyzed for 3 days against distilled water and freeze-dried.

Preparation of SDS Subunits

The solutions used for the reduction of glutenin and SDS-PAGE are listed in Table II. Glutenin (10 mg.) was shaken overnight at 40°C. in 1 ml. of protein solvent containing 1% (w./v.) SDS and 1% (v./v.) β-mercaptoethanol. After reduction and complexing with SDS, the protein solutions were made 10% w./v. in sucrose, and 20 μl. of a 0.3% aqueous solution of bromophenol blue was added to each sample. Molecular-weight markers were complexed with SDS at a concentration of 1 mg. per ml. Since reduction of the standard proteins was not required, 0.002M N-ethylmaleimide replaced the β-mercaptoethanol for all marker proteins.

Preparation of Gels

The 5% gel was prepared by dissolving 11.0 g. of acrylamide and 0.29 g. of bisacrylamide cross-linking agent in 210 ml. of electrode buffer at pH 7.3 containing 0.33 ml. of N,N,N′,N′-tetramethylethylenediamine. This solution was deaerated, and 100 mg. of ammonium persulfate in 10 ml. of buffer was added. The gel slab was polymerized in an E-C vertical gel electrophoresis unit using 6-mm. spacers and an eight-slot mold.

TABLE II. SOLUTIONS FOR SDS-PAGE

Stock Buffer: 1 liter containing 7.8 g. $NaH_2PO_4 \cdot H_2O$, 20.4 g. Na_2HPO_4, and 10.0 g. SDS

Electrode Buffer:
Dilute the stock buffer to one-tenth original strength. Final pH 7.3

Protein Solvent:
The electrode buffer containing 1% (w./v.) of SDS and 1% (v./v.) of β-mercaptoethanol

Electrophoresis

After addition of the buffer and removal of the slot former, the gel was conditioned for 30 min. using the voltage required for the electrophoretic experiment. Fifty microliters of the SDS-glutenin solution, or 10 μl. of the standard protein solution, was added to each slot and allowed to settle for 10 min. with the buffer pump turned off. The protein-SDS complexes were then run into the gel at 130 v. (120 ma.) and, after 10 min., the buffer pump was turned on. The run was continued at 130 v. until the bromophenol blue marker had traveled 7 cm. (approximately 3 hr.). On completion of electrophoresis, the gel was stained with Coomassie Brilliant Blue according to the method of Koenig et al. (10). After destaining, the gels were photographed and the mobilities of the protein bands were measured relative to the bromophenol blue marker. A plot of log of molecular weight versus mobility for the proteins of known molecular weight gave a calibration curve that was used to determine the molecular weightsof the glutenin subunits. This plot showed a slight downward curvature as found by Bietz and Wall (6) for the same (5%) gel concentration. SDS-PAGE yields molecular weights with an accuracy of better than ±10% for polypeptides with molecular weights between 15,000 and 100,000 (11).

RESULTS AND DISCUSSION

Varietal Variation in the Subunit Molecular-Weight Distribution of Glutenin

Since the major objective of our study was to determine if any relationship existed between breadmaking quality and the glutenin subunits, the first electrophoretic runs were on reduced glutenins from the 26 flours arranged in order of decreasing loaf-volume per unit protein (see Table II of reference 1). The electropherograms are shown in Fig. 1.

Two facts are apparent from the results of Fig. 1. Firstly, there is no obvious characteristic SDS-PAGE pattern of glutenin subunits for a particular level of baking quality. Secondly, although there are obvious differences between varieties, all showed the characteristic pattern of hexaploid bread wheats as found by Bietz and Wall (6). These patterns can be divided into three fairly distinct regions: High molecular weight (greater than 100,000 daltons), intermediate (60,000), and low-molecular-weight subunits (less than 30,000). All glutenins examined in the present study contained a number of subunits in each of these regions.

Most variation was noted in the number and molecular weight of the slow-moving or high-molecular-weight subunits. The number of bands varied from three to five in this region. Variation was also evident in the fast-moving subunits, but this was not as pronounced as in the high-molecular-weight subunits.

Since the glutenins from these wheats do not have a subunit molecular-weight distribution characteristic of their breadmaking quality, it was concluded that additional factors are involved in the determination of the overall quality. The absence of high-molecular-weight glutenin subunits in durum wheats (4,6) indicates that their presence in bread wheats may be a necessary condition for quality. However, the results obtained in the present study indicate it is not a sufficient condition.

To illustrate this conclusion, the reduced glutenins of two closely related varieties of very different baking quality (Thatcher and Thatcher backcross) and two genetically unrelated varieties of similar quality (Opal and Fortuna) were

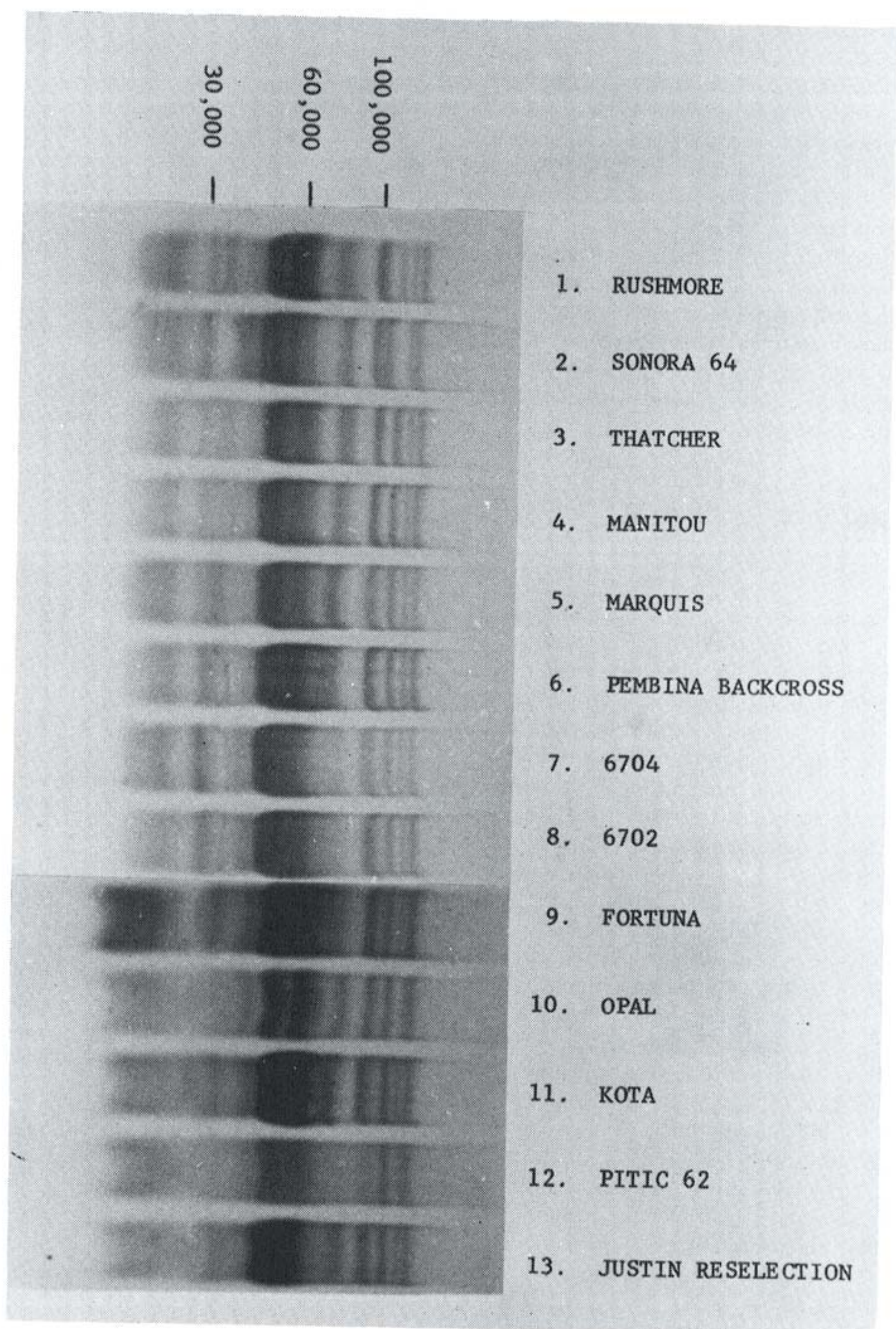

Fig. 1. SDS-PAGE patterns of the glutenin subunits of 26 bread wheats. The varieties are arranged in order of decreasing baking quality from 1 to 26.

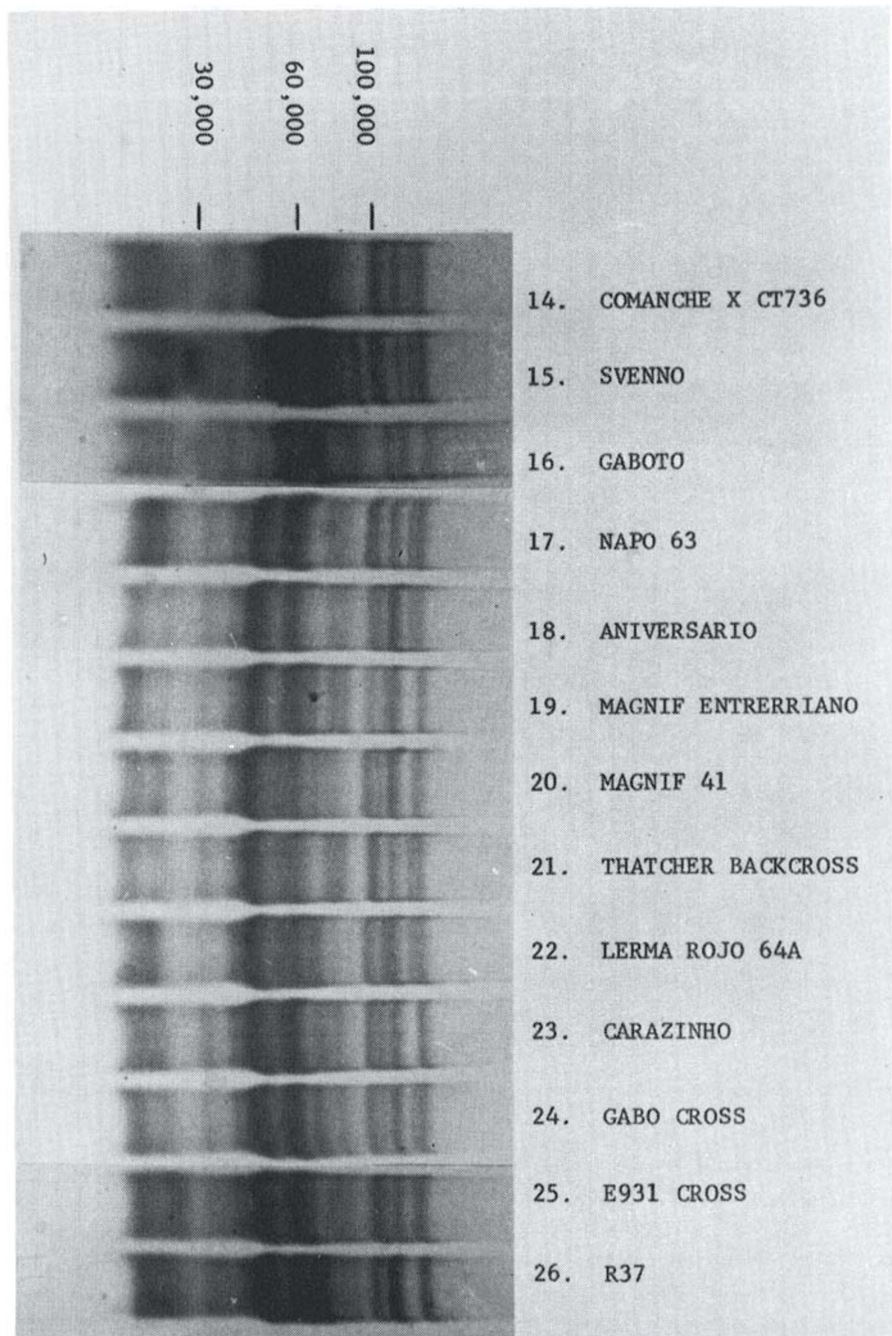

Fig. 1. Continued.

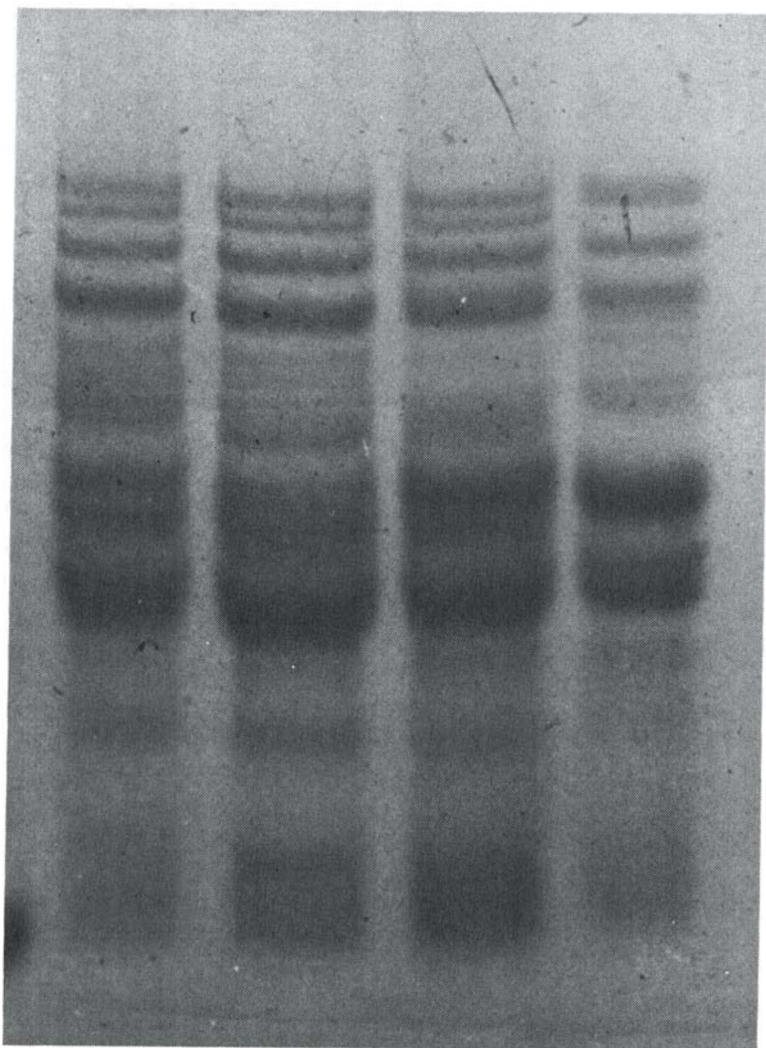

Fig. 2. SDS-PAGE patterns of the glutenin subunits of Thatcher, Thatcher backcross, Fortuna, and Opal.

compared by SDS-PAGE on the same gel slab (Fig. 2). Although Thatcher and its backcross were distinctly different in baking quality, their patterns were qualitatively identical. On the other hand, Fortuna and Opal, of essentially equal quality, had very different patterns.

The 26 varieties used in this study included many that are related to the variety Marquis. These varieties, with their number referring to their order in Fig. 1 given in parentheses, are: Rushmore (1), Thatcher (3), Manitou (4), Marquis (5), Pembina backcross (6), 6702 (7), 6704 (8), Fortuna (9), Justin reselection (13), and

Thatcher backcross (21). The glutenin subunits of all these varieties were qualitatively identical by SDS-PAGE, indicating that the subunits of glutenin are genetically controlled.

Effect of Location of Growth on the Subunit Molecular-Weight Distribution of Glutenin

The reduced glutenins of four varieties of widely different baking quality (Rushmore, Manitou, Pitic 62, and R37), each grown at four stations, were examined by SDS-PAGE. No qualitative or obvious quantitative inter-station differences were found for any of the varieties. It was therefore concluded that the molecular-weight distribution of glutenin subunits of healthy bread wheats are not affected by location of growth.

SUMMARY

The number of distinct subunits and the molecular weight distribution of subunits of reduced glutenin are genetically controlled and are not affected by location of growth. There is no apparent relationship between the SDS-PAGE patterns of the reduced glutenins and breadmaking quality.

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Literature Cited

1. ORTH, R. A., and BUSHUK, W. A comparative study of the proteins of wheat of diverse baking quality. Cereal Chem. 49: 268 (1972).
2. ORTH, R. A., BAKER, R. J., and BUSHUK, W. Statistical evaluation of techniques for predicting baking quality of wheat cultivars. Can. J. Plant Sci. 52: 139 (1972).
3. WOYCHIK, J. H., HUEBNER, F. R., and DIMLER, R. J. Reduction and starch-gel electrophoresis of wheat gliadin and glutenin. Arch. Biochem. Biophys. 105: 151 (1964).
4. HUEBNER, F. R. Comparative studies on glutenins from different classes of wheat. Agr. Food Chem. 18: 256 (1970).
5. SHAPIRO, A. L., VINUELA, E., and MAIZEL, J. F., Jr. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28: 815 (1967).
6. BIETZ, J. A., and WALL, J. S. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49: 416 (1972).
7. CHEN, C. H., and BUSHUK, W. Nature of proteins in *Triticale* and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50: 9 (1970).
8. MITA, T., and YONEZAWA, D. Separation of component polypeptides of glutenin by isoelectric focusing. Agr. Biol. Chem. 35: 1792 (1971).
9. ORTH, R. A., and BUSHUK, W. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50:106 (1973).
10. KOENIG, R., STEGEMANN, H., FRANCKSEN, H., and PAUL, H. L. Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207: 184 (1970).
11. WEBER, K., and OSBORN, M. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406 (1969).

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