Studies of Glutenin. IV. Microscopic Structure and Its Relations to Breadmaking Quality[1]

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ABSTRACT

Glutenins of two varieties of hard red spring wheat, Canthatch and Manitou, one variety of durum wheat, Stewart 63, a synthetic hexaploid (AABBDD) wheat and its tetraploid (AABB) and diploid (squarrosa, DD) parents, and one variety of spring rye, Prolific, were analyzed by scanning electron microscopy. The glutenins of Canthatch, Manitou, and the synthetic hexaploid were fibrous in structure, whereas those of Stewart 63 and Tetracanthatch were characterized by ribbonlike and film structures. Rye glutenin showed characteristic rodlike structures. *Squarrosa* glutenin appeared fibrous like the glutenin of the bread wheats and the synthetic hexaploid. The observed differences in microscopic structure can be related to glutenin elasticity. Glutenin of Manitou was fibrous and of uniform structure. On reduction of its disulfide bonds, this glutenin completely lost its fibrous structure.

Although the electron microscope has been used in studies of the wheat kernel, flour, dough, and starch (1-6) there is only one report (2) in the literature of a study of glutenin from one sample of wheat. Because of the very important role of this protein in the breadmaking quality of bread wheats, it was felt that its microscopic structure might show a recognizable relationship to its ability to contribute to the formation of the gluten complex that is required for baking quality.

Some results on the structure of gliadin and glutenin were published by Seckinger and Wolf (2). In films cast from dispersions of these proteins, gliadin showed filmlike structures, whereas glutenin formed strands.

This article presents scanning electron microscope (SEM) results on glutenin from a number of related cereal grains, and discusses the possible relation of the microscopic structure to breadmaking quality.

MATERIALS AND METHODS

Grain Samples

The grain samples used are listed below. All were grown on experimental plots at the University of Manitoba.

Variety, etc.	*Type or Class*	*Chromosome Number*	*Genomic Constitution*
Canthatch	hard red spring wheat	2n=6x=42	AABBDD
Manitou	hard red spring wheat	2n=6x=42	AABBDD
Synthetic hexaploid	unclassified	2n=6x=42	AABBDD
Tetracanthatch	tetraploid derived from Canthatch	2n=4x=28	AABB
Stewart 63	amber durum wheat	2n=4x=28	AABB
Prolific	spring rye	2n=2x=14	RR
Strangulata	*aegilops squarrosa*	2n=2x=14	DD

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Glutenin Preparation

Glutenins were prepared by pH precipitation after extraction of the protein with AUC (0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide) (7). AUC extracts were prepared from ground grain of Canthatch, Tetracanthatch, *squarrosa*, synthetic hexaploid, Prolific (rye) and Stewart 63 (durum), and from washed-out gluten of Manitou.

Reduction of Glutenin

Manitou glutenin (50 mg.) was dispersed in 10 ml. of 0.1M phosphate buffer of pH 8.0 containing 1% (v./v.) β-mercaptoethanol and 6M urea. The dispersion was shaken overnight at 40°, dialyzed against deionized water for 6 days, and freeze-dried. A control sample was subjected to the same treatment except the β-mercaptoethanol was omitted.

Scanning Electron Microscopy

Freeze-dried glutenin material was attached to circular stubs with double-sided

Fig. 1. SEM micrograph of purified glutenin of the bread wheat variety Manitou.

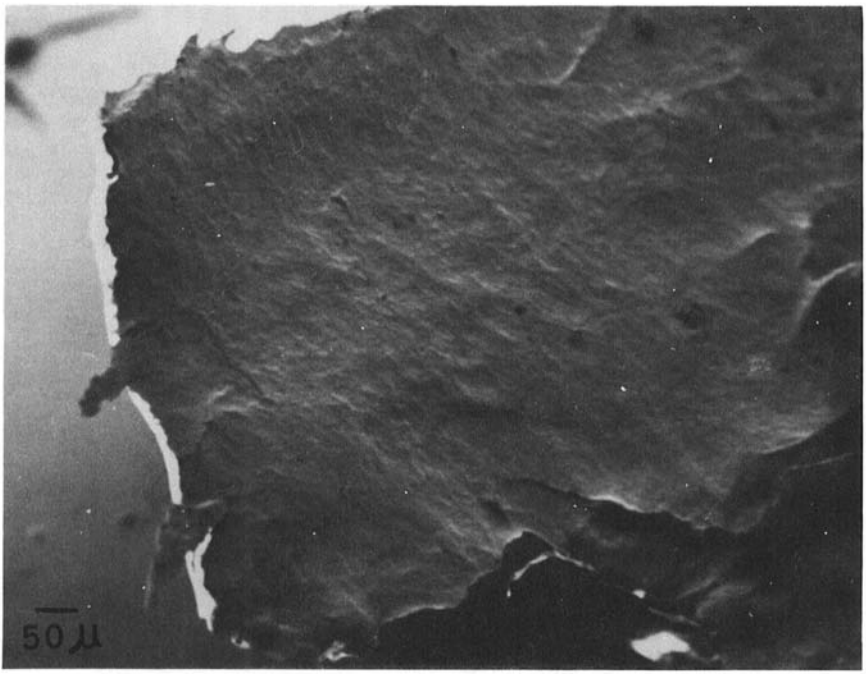

Fig. 2. SEM micrograph of reduced glutenin of the bread wheat variety Manitou.

tape and coated with gold to a thickness of 20 to 25 nm. The mounted specimens were examined in a Cambridge "Stereoscan" MK IIa scanning electron microscope at an accelerating potential of 10 kv. Samples were viewed by scanning the total specimen, and a representative area was photographed on 35-mm. Kodak Panatomic X film.

RESULTS AND DISCUSSION

Glutenin of Manitou (Fig. 1) showed a characteristic fibrous structure with many thick strands (10 μ diameter) intertwined with thin strands (1 μ). The strands were generally aligned, giving the impression of a unidirectional organization of the fibers. None of the micrographs of this glutenin showed any starch granules that would be readily identifiable because of their characteristic size, shape, and structure. In cross-section, the fibers appear circular and there is considerable evidence of filmy material between the strands, especially at points of contact.

Reduction of the Manitou glutenin produced a dramatic change in its structure (Fig. 2). All of the original fibrous structure was lost. The reduced glutenin had no distinguishing or regular features. The structural change observed is a conversion of a regular fibrous structure to one that appears completely amorphous. Presumably, this structural change would lead to a marked change in the rheological properties of the hydrated glutenin. Fundamentally, one would expect the characteristic elasticity of bread wheat glutenins to be completely lost when the structure of Fig. 1 is converted to that of Fig. 2.

The fact that the reduced glutenin lacked the fibrous structure of the control indicates that the structures observed are not due to sample preparation alone but depend on the inherent nature of the proteins. This conclusion is supported by results obtained in another study (8) in which proteins from Manitou flour, fractionated on Sephadex G-100 in the solvent AUC, showed microscopic structures dependent on their molecular weight. The structure of the low-molecular-weight proteins was very different to those of high molecular weight.

Glutenin of Canthatch (Fig. 3) appeared as stringy intertwined fibers. In addition, it contained some flat filmy material. In structure, this glutenin appeared slightly different from the Manitou glutenin. Presumably this alteration of structure is due to the minor difference in the preparative methods used; however, the typical fibrous structure of bread wheat glutenin is still quite evident. Formation of a gluten ball in the preparation of Manitou glutenin (Fig. 1) may be responsible for the parallel alignment of the glutenin fibers. Canthatch flour, which did not undergo gluten ball formation, yielded a glutenin with less fibrous structure than

Fig. 3. SEM micrograph of purified glutenin of the bread wheat variety Canthatch.

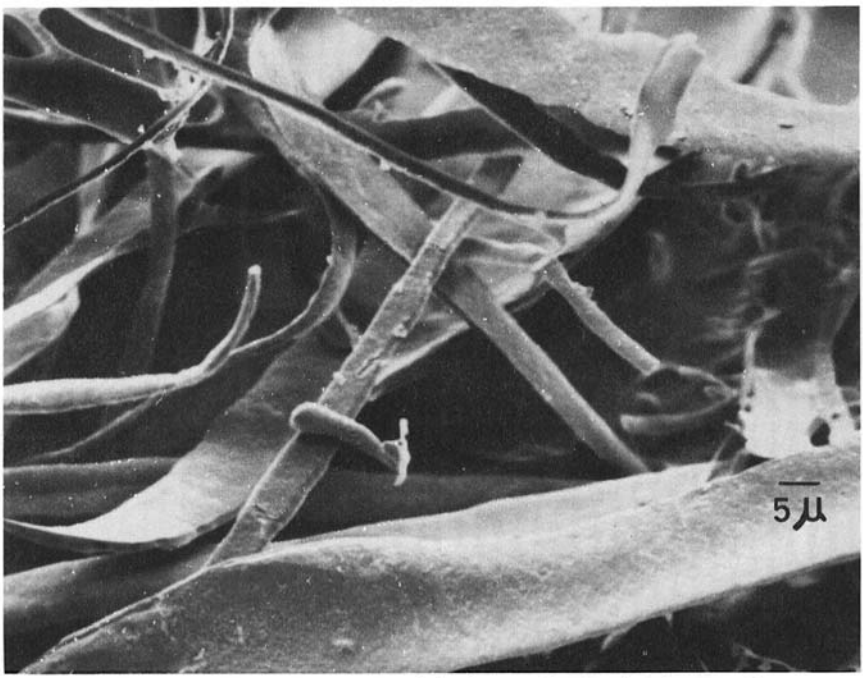

Fig. 4. SEM micrograph of purified glutenin of the durum wheat variety Stewart 63.

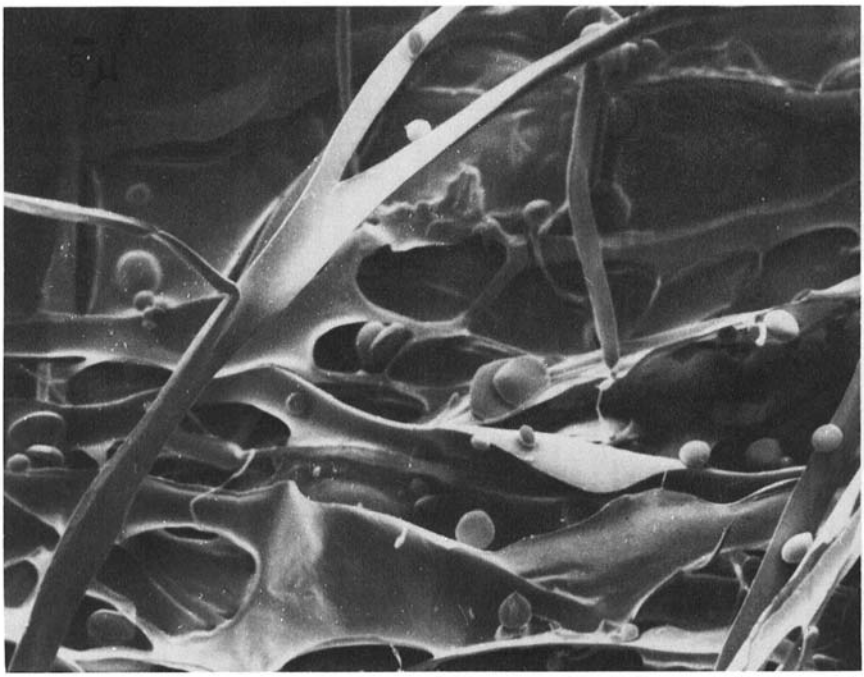

Fig. 5. SEM micrograph of purified glutenin of the extracted AABB tetraploid wheat variety Tetracanthatch.

Manitou glutenin but considerably more than that of the AABB tetraploid wheats discussed below.

Figure 4 is a micrograph of the glutenin of the durum wheat, Stewart 63. In contrast to the bread wheat glutenin, the durum glutenin is characterized by flat ribbonlike structures and contains a large proportion of filmy material. When these results are considered relative to functional properties, the durum glutenin would be considerably less elastic than the glutenins of the bread wheats shown in Figs. 1 and 3. The microscopic structural features are generally consistent with the well-known rheological properties of bread and durum wheat doughs.

The results presented above suggested a possible relationship between the observed microscopic structure of glutenin and the functional properties of doughs derived from the same wheat. Accordingly, a SEM study of the glutenins of a synthetic hexaploid bread wheat and of the tetraploid and diploid parents was carried out to obtain further information on this relationship.

Figure 5 is a micrograph of the glutenin of Tetracanthatch. This glutenin had very little fibrous structure and consists largely of layered ribbonlike structures. In this respect, Tetracanthatch glutenin was similar to the glutenin from the natural durum wheat (tetraploid), Stewart 63. Also evident in the micrograph of Fig. 5 are discrete disc-shaped particles approximately 5 to 10 μ in diameter. These are probably small starch granules.

Removal of the D-genome from Canthatch produced a dramatic change in the

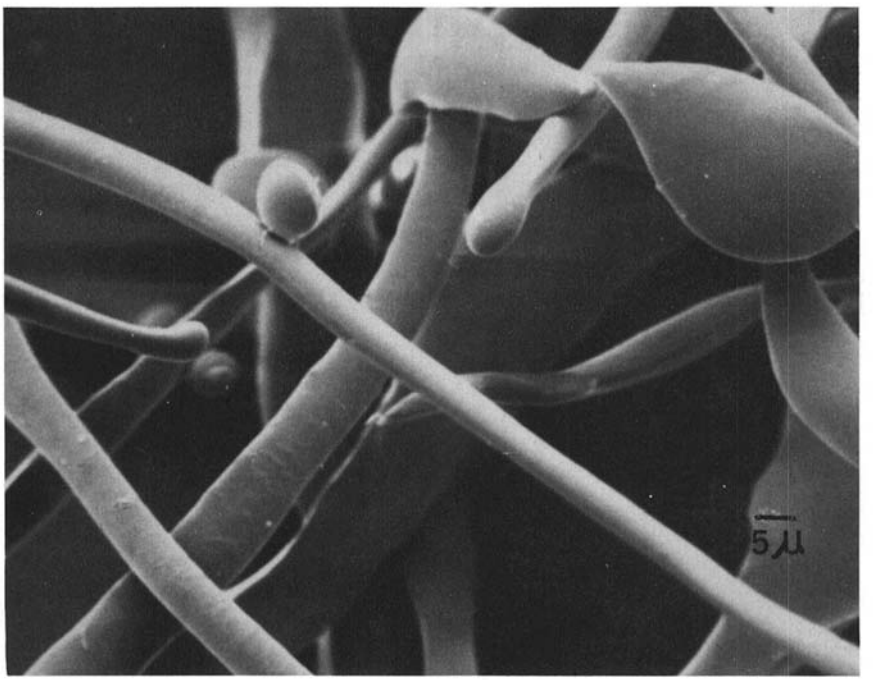

Fig. 6. SEM micrograph of purified glutenin of the synthetic hexaploid wheat Tetracanthatch X *aegilops squarrosa* variety *strangulata*.

microscopic structure of the glutenin. It completely lost its fibrous appearance and became quite amorphous. In breadmaking quality, Tetracanthatch was inferior to Canthatch (9). The loss of baking quality parallels the change in structure of the glutenin.

Addition of the D-genome from *aegilops squarrosa* variety *strangulata* to Tetracanthatch to produce a synthetic AABBDD hexaploid wheat gave a noticeable improvement in breadmaking quality (9). The glutenin of the synthetic hexaploid (Fig. 6) was considerably more fibrous than that of Tetracanthatch with strands of similar diameter and shape as those of Canthatch glutenin.

The micrograph of the glutenin of the *strangulata* variety of *ae. squarrosa* (progenitor of the D-genome of bread wheats) (Fig. 7) had the characteristic fibrous structure typical of bread wheats. Small amounts of sheetlike material and small starch granules were also evident in the *ae. squarrosa* glutenin preparation examined. The change of the ribbonlike glutenin of Tetracanthatch to the fibrous glutenin of the synthetic hexaploid can be attributed to the D-genome that was added.

Glutenin of the one variety of rye that was examined (Fig. 8) showed characteristic short rodlike structures, but essentially no thin fibers. Very little entanglement of the rodlike particles of this glutenin was observed in micrographs covering large areas of this protein. Rye glutenin had a structure quite distinct from

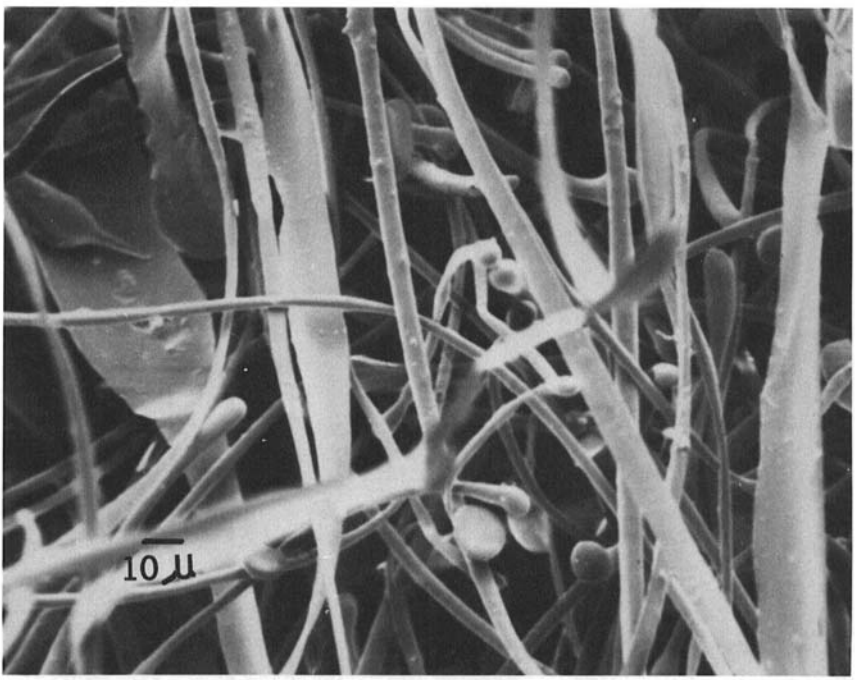

Fig. 7. SEM micrograph of purified glutenin of *aegilops squarrosa* variety *strangulata* (D-genome, diploid).

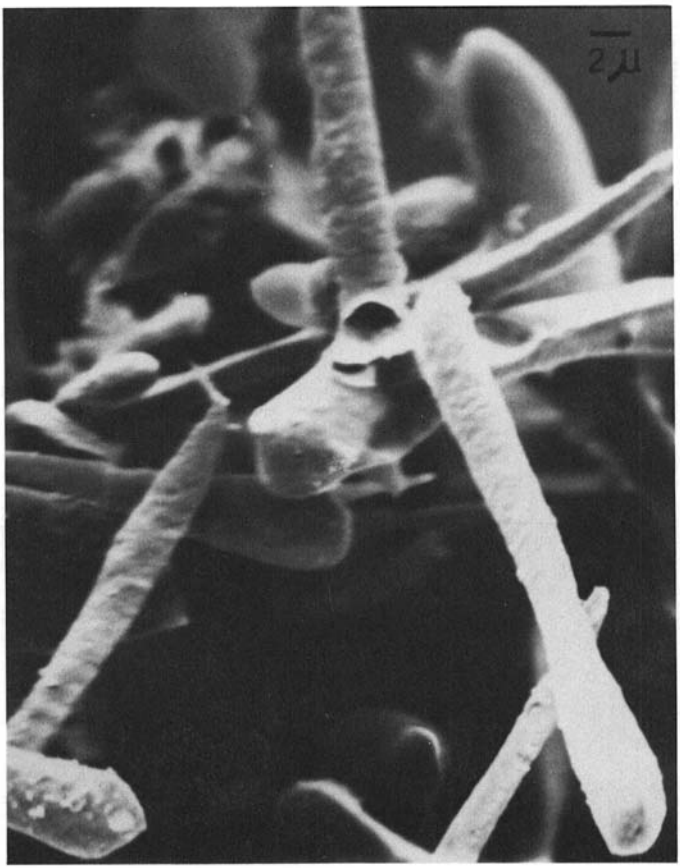

Fig. 8. SEM micrograph of purified glutenin of the spring rye variety Prolific.

the structures of the other glutenins examined in this study. In relation to functional properties, it would be expected that the elasticity of a structure such as shown in Fig. 8 would be considerably less than that of the structures in Figs. 1,3,6, and 7.

Results presented in this article showed that the SEM can be used to advantage in the determination of the microscopic structure of glutenin from various grain species. Furthermore, the structure of glutenin from bread wheats can be readily distinguished from that of other wheats and grains that do not have the functional properties that are required for breadmaking quality.

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