Development and "Undevelopment" of Wheat Dough by Mixing: Microscopic Structure and Its Relations to Bread-Making Quality[1]

O. PAREDES-LOPEZ[2] and W. BUSHUK, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

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ABSTRACT

Scanning electron microscopy (SEM) of dough revealed changes in dough microstructure during development and undevelopment. There was evidence that undevelopment transformed the gluten of optimally developed dough from a continuous membranelike structure into a discontinuous meshlike structure. SEM of gliadins and glutenins isolated from doughs showed structural differences between mixing treatments. Undevelopment transformed glutenins from a fibrillar structure into one that contained ruptured membranes and numerous particles or globules. On the basis of the SEM results and other analytical results, we postulated that undevelopment results in the association of gluten proteins (mainly glutenin) into aggregates. This structural change transformed the membranelike structure of optimally developed gluten with good gas retention capacity, into a fibrillar or globular structure with poor gas retention capacity. The exact nature of the forces involved in the aggregation process remains to be discovered.

Scanning electron microscopy (SEM) has been used in recent years to study the microscopic structure of wheat-flour doughs and the protein fractions isolated from them (Bernardin and Kasarda 1973; Crozet 1977; Orth et al 1973a, 1973b; Tu and Tsen 1978). Advantages of SEM are a simpler method of sample preparation than transmission electron microscopy, and higher possible magnifications than light microscopy.

The purpose of this study was to examine the microstructure of developed and undeveloped doughs, and the gliadins and glutenins isolated from them for any differences in structure that might be correlated to the bread quality.

MATERIALS AND METHODS

The dough samples obtained with various mixing treatments (development, undevelopment, etc.) from three flours of different strength are the same as those described previously (Paredes-Lopez and Bushuk 1982). Samples from treatments 2 (developed dough), 5 (16 min of undevelopment), 6 (redevelopment), and 8 (overmixed) were used to examine structural changes in dough. To examine the inner surfaces of the doughs, small pieces of dough were frozen in liquid nitrogen and fractured before and after freeze-drying. The former specimens will be referred to as "fractured-while-frozen" (FWF) and the latter as "fractured-after-freeze-drying" (FAFD).

Gliadins and glutenins examined in this study were isolated from freeze-dried doughs (samples 2 and 5) by the modified Osborne fractionation procedure (Chen and Bushuk 1970). Freeze-drying was the technique selected for drying because it causes the least disturbance of dough structure (Varriano-Marston 1977).

For microscopy, specimens were attached to the sample stubs with silver conducting paint and coated with a layer of gold approximately 20–25 nm thick in a Balzers sputter coater. The coated samples were viewed in a Cambridge "Stereoscan" MK IIa scanning electron microscope at an accelerating potential of 10 kV; representative areas were photographed on 35-mm Kodak Panatomic X film (Dexter et al 1979).

RESULTS AND DISCUSSION

SEM Studies of Dough Structure

The surfaces of the FWF specimens showed many cleaved starch granules (Fig. 1, Glenlea; Fig. 2, Neepawa; and Fig. 3, Fredrick/Neepawa (Fr/Np) doughs). The optimally developed doughs of Glenlea (Fig. 1A) and of Neepawa (Fig. 2A) showed a continuous interconnected gluten matrix surrounding most of the starch granules. Undeveloped doughs of Glenlea (Fig. 1B) and Neepawa (Fig. 2B) exhibited discontinuous gluten structure. Undevelopment apparently destroys the continuous gluten network. The gluten matrix of remixed Glenlea dough was interconnected (Fig. 1C). It surrounded the starch granules in a manner similar to that in developed dough (Fig. 1A). In the weaker, Neepawa flour, remixing did not recreate a continuous gluten matrix (Fig. 2C) analogous to that in optimally developed dough (Fig. 2B).

The continuous gluten matrix of the developed dough was disrupted by the extended mixing, as shown in the overmixed Neepawa dough (Fig. 2D). The analogous dough of Glenlea (Fig. 1D) did not show this disruption.

FWF samples of Fr/Np doughs (weakest flour) exhibited for all mixing treatments a discontinuous gluten network (Fig. 3A–D). The starch granules appeared to be only partially covered by the gluten membrane. These doughs showed evidence of what appeared to be ruptured gluten membranes with many open areas.

FAFD specimens were examined at higher magnifications so that their microstructure could be examined in more detail. In optimally developed Glenlea dough, starch granules were

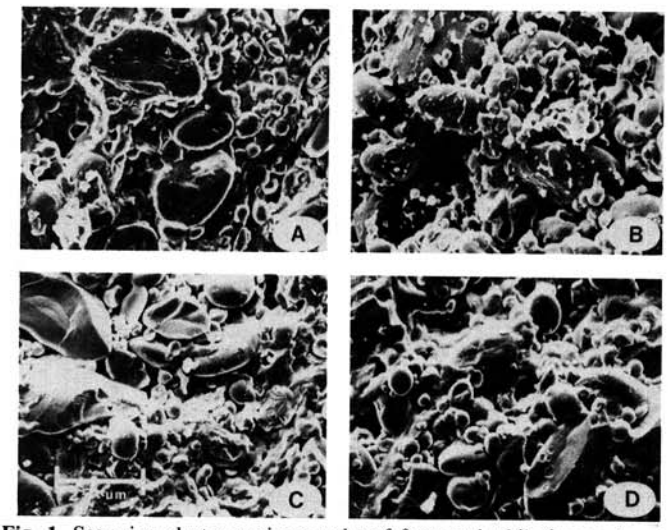

Fig. 1. Scanning electron micrographs of fractured-while-frozen dough samples of Glenlea. **A**, developed dough (sample 2); **B**, undeveloped dough (sample 5); **C**, redeveloped dough (sample 6); and **D**, overmixed dough (sample 8).

[1]Presented at the AACC 65th Annual Meeting, Chicago, IL, September 1980. Contribution 596 from the Department of Plant Science, University of Manitoba.
[2]Present Address: CIEA del Instituto Politecnico Nal., Unidad Irapuato, Apdo. P. 629, 36500 Irapuato, Gto., Mexico.

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surrounded by gluten membranes. The dough surfaces were uniform (Fig. 4A), as observed by Khoo et al (1975), Moss et al (1979), and Stenvert et al (1979). SEMs for the undeveloped Glenlea dough were distinctly different from those for the two other flours (Fig. 4B). Glenlea dough contained many broken gluten strands. Gluten membranes appeared to be ruptured and separated from the starch granules, suggesting a decrease in the protein-starch interaction. A change of the gluten matrix was evident when the results for the optimally developed and undeveloped doughs were compared. Some small starch granules appeared to be still embedded in the gluten (Fig. 4B).

The SEM photograph of overmixed Glenlea dough (Fig. 4C) shows that most of the starch granules are still covered by a gluten membrane. This property of Glenlea dough might be related to its high mixing stability. The gluten network of the undeveloped dough appears to be less uniform than that of optimally developed (Fig. 4A) and overmixed (Fig. 4C) doughs. Because a less uniform gluten matrix might retain gas poorly, these structural features are in general agreement with the observation that the loaf volume of bread from undeveloped Glenlea dough was lower than that obtained from the optimally developed and undeveloped doughs (Paredes-Lopez and Bushuk 1982).

Figure 5 shows the microstructure of FAFD samples of Neepawa dough from three mixing treatments: optimally developed (Fig. 5A); undeveloped (Fig. 5B); and overmixed (Fig. 5C). A comparison of the structure of optimally developed and overmixed doughs with that of undeveloped dough shows the deleterious effects of the latter mixing treatment. In the undeveloped dough, most of the starch granules have bits of membrane (gluten) adhering to them.

For the very strong (Glenlea) and strong (Neepawa) flours, SEM photographs of overmixed doughs showed that the gluten structure was no longer continuous (Figs. 4C and 5C). The breakdown of the gluten membranes, as indicated by SEM, accompanies the decrease of loaf volume of the bread produced from overmixed doughs (Paredes-Lopez and Bushuk 1982).

The micrographs of FAFD samples of Fr/Np dough are not shown because, in this case, no distinct features were observed for the various mixing treatments. As indicated previously (Paredes-Lopez and Bushuk 1982), the effect of undevelopment on loaf volume was less pronounced for this weaker flour than for the two stronger flours.

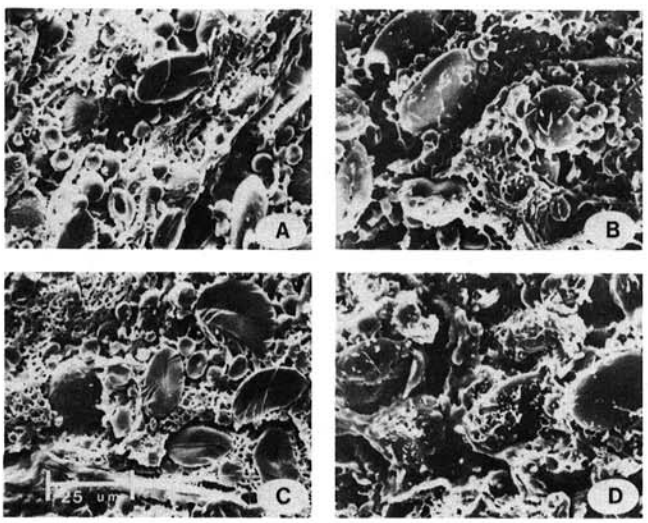

Fig. 2. Scanning electron micrographs of fractured-while-frozen dough samples of Neepawa. **A,** developed dough (sample 2); **B,** undeveloped dough (sample 5); **C,** redeveloped dough (sample 6); and **D,** overmixed dough (sample 8).

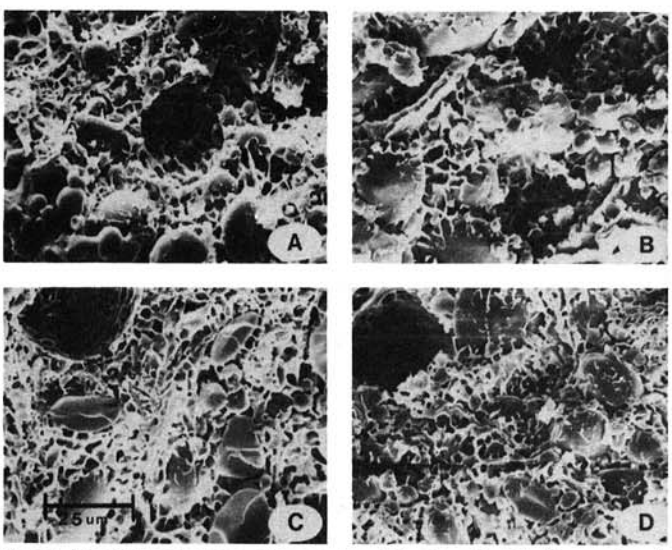

Fig. 3. Scanning electron micrographs of fractured-while-frozen dough samples of Fredrick/Neepawa (50/50). **A,** developed dough (sample 2); **B,** undeveloped dough (sample 5); **C,** redeveloped dough (sample 6); and **D,** overmixed dough (sample 8).

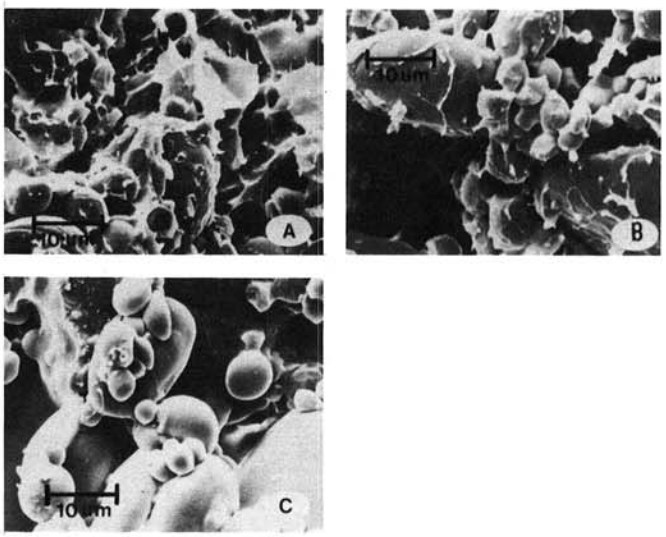

Fig. 4. Scanning electron micrographs of Glenlea doughs fractured after freeze-drying. **A,** developed dough (sample 2); **B,** undeveloped dough (sample 5); and **C,** overmixed dough (sample 8).

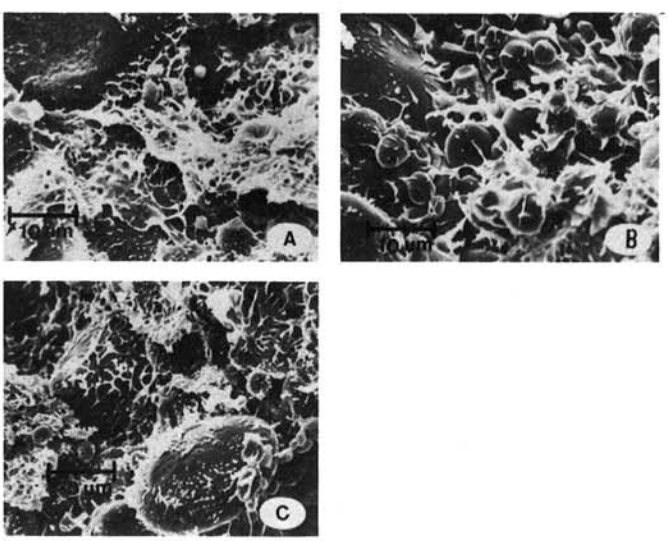

Fig. 5. Scanning electron micrographs of Neepawa doughs fractured after freeze-drying. **A,** developed dough (sample 2); **B,** undeveloped dough (sample 5); **C,** overmixed dough (sample 8).

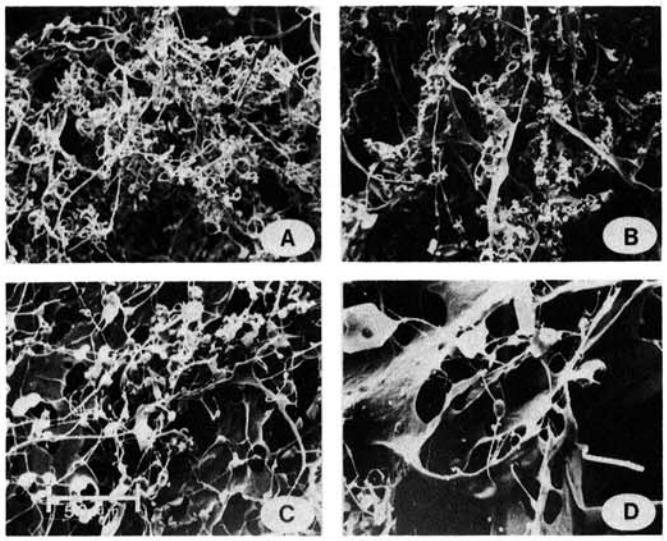

Fig. 6. Scanning electron micrographs of gliadins and glutenins isolated from Glenlea doughs. **A,** gliadin from developed dough (sample 2); **B,** gliadin from undeveloped dough (sample 5); **C,** glutenin from developed dough (sample 2); **D,** glutenin from undeveloped dough (sample 5).

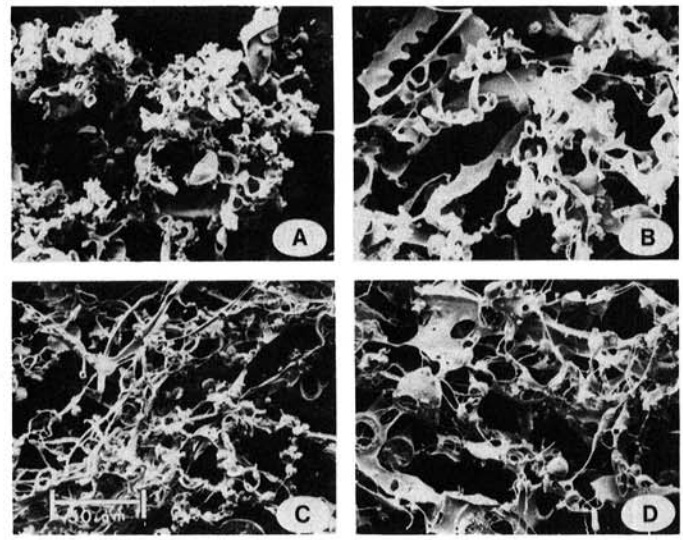

Fig. 7. Scanning electron micrographs of gliadins and glutenins isolated from Neepawa doughs. **A,** gliadin from developed dough (sample 2); **B,** gliadin from undeveloped dough (sample 5); **C,** glutenin from developed dough (sample 2); **D,** glutenin from undeveloped dough (sample 5).

SEM Studies of Gliadin and Glutenin Protein Fractions

Gliadin and glutenin fractions from optimally developed and undeveloped doughs were selected for SEM examination on the assumption, based on previous results (Paredes-Lopez and Bushuk 1982), that they might show changes offering an explanation for the undevelopment phenomenon. The gliadin and glutenin fractions of optimally mixed Glenlea dough (Fig. 6A and C, respectively), showed a highly fibrous structure. The average diameter of gliadin and glutenin fibrils was about 2 μm. The gliadin, however, did not contain the sheet or filmlike structures present in the glutenin. Undevelopment produced changes in the appearance of both (Fig. 6B and D, respectively). The structure appeared to be less fibrous and the diameter of gliadin fibrils increased to about 5 μm. In addition, the glutenin fraction showed predominantly sheetlike structures. These structures ranged from 5 to 50 μm.

The gliadin and glutenin fractions of the optimally developed dough of Neepawa (Fig. 7A and C, respectively) showed numerous small spherical particles 2–4 μm in diameter. The gliadin did not show any of the fine (2–3 μm in diameter) fibrils present in the glutenin. In contrast, the gliadin appeared as strands intermixed with numerous spherical particles. The small particles of glutenin seemed to be linked by fibrils. Undevelopment produced a significant change in the gliadin and glutenin microstructure (compare Fig. 7A and B and Fig. 7C and D). Both fractions contained larger (6–12 μm) spherical globules than the corresponding spherical fractions from the optimally developed dough. Spherical particles were also observed in SEM of glutenin by Kaczkowski (1979) and Orth et al (1973a, 1973b).

GENERAL DISCUSSION

Our results suggest that flour strength is related to structural changes occurring during undevelopment. Both doughs and isolated protein fractions showed structural changes. On the basis of the SEM results, we have postulated that undevelopment promotes the conversion of the continuous membranous structure of gluten into discontinuous fibrillar and globular structures. The results obtained from the gliadin and glutenin fractions are consistent with this hypothesis. Furthermore, the hypothesis is also qualitatively consistent with the baking and solubility fractionation results using the same flours (Paredes-Lopez and Bushuk 1982).

Results presented here and elsewhere (Paredes-Lopez and Bushuk 1982) suggest that gliadin and glutenin molecules associate or dissociate according to the mixing treatment. Both protein classes, but especially glutenins, showed a marked tendency to associate during undevelopment to form aggregates. Microscopic evidence of the aggregation of gluten proteins was published by Bernardin (1978). We have postulated that two opposing processes occur during mixing: the gradual transformation by aggregation of hydrated flour proteins into continuous membranes; and the breakdown of these membranes by overmixing or by aggregation, which appears to be facilitated by mixing speeds less than those required for development. The minimum critical mixing speed depends on quality (Tipples and Kilborn 1975). Bread of optimum quality results when balance between membrane formation and disruption is attained. This is the point of optimum dough development. The above explanation is speculative. Further research is needed on the specific nature of the forces that cause gluten proteins to form membranes or to aggregate (on undevelopment) into relatively insoluble discontinuous particles.

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