BREADMAKING STUDIED BY LIGHT AND TRANSMISSION ELECTRON MICROSCOPY[1]

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ABSTRACT

The structure of a water-flour dough, a fermented dough containing all bread ingredients (complete dough), and baked bread was examined by light and transmission electron microscopy. Protein strands provided a matrix network in a mixed dough; matrix formation required adequate mixing. Excessive mixing destroyed the matrix. The effect of overmixing and undermixing depended on mixing requirements and mixing tolerance of the flour. Fermentation of a complete dough produced gas vacuoles. After oven spring, protein strands were thin and had small vacuoles. Starch granules in the bottom center of a loaf varied widely in degree of gelatinization after oven spring; starch started to gelatinize from the interior of the granule and appeared fibrous. In the baked bread, most of the starch was gelatinized into fibrous strands interwoven with thin protein strands.

The structure of wheat-flour doughs and the development of the doughs into bread have been studied by the use of transmission electron microscopy (TEM) by Simmonds (1) and Khoo *et al.* (2). Sandstedt *et al.* (3) pointed out difficulties in the preparation of thin sections for examination by light microscopy (LM); preparation of dough and very thin sections for TEM is much more difficult. Simmonds (1) described some changes that occurred during the conversion of flour to dough and suggested that two types of inclusions occurred in the protein phase of the dough: Type I inclusions were irregular, stained densely, and presumably had been formed from the endoplasmic reticulum. Type II inclusions were spherical, had not been formed in doughs from defatted flours, and therefore were thought to be lipid rich. Khoo *et al.* (2) briefly described various stages of breadmaking—freshly mixed dough, fermented and proofed dough, and fully baked bread. During baking, the protein fraction changed little (in microscopically visible structures), but the starch granules, particularly the large ones, became gelatinized.

In this study we used LM and TEM to 1) elucidate microscopic changes associated with undermixed, optimally mixed, and overmixed flour-water doughs, 2) determine differences among doughs formed from poor- and good-quality flours, and 3) examine the ultrastructural changes that take place in a dough containing all bread ingredients during mixing, fermentation, proofing, and baking.

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MATERIALS AND METHODS

Two composite flours and flours from individual wheat cultivars were milled from hard red winter wheats. Regional Baking Standards (RBS) were composites of many wheat varieties grown in many locations throughout the Great Plains in 1974 and 1975; CI 12995 was a good-quality, long-mixing flour, and KS 501099 was a poor-quality, short-mixing flour (Table I). Grossly undermixed, optimally mixed, and grossly overmixed doughs were made from 10 g of flour (RBS-74, KS 501099, CI 12995) and optimum water on a National Mfg. Co. (Lincoln, NE) mixer (4). Grossly undermixed and overmixed doughs resulted, respectively, from mixing 50% below and 50% above the optimal mixing time.[5]

Bread was made from RBS-75 flour (100 g) according to the procedure of Shogren *et al.* (5). Moisture, protein, and ash were determined by AACC Approved Methods 44-15A, 46-11, and 08-01, respectively (6). Small samples (1-mm^3 pieces) were dissected from the doughs with forceps. Dough and bread were sampled after optimal mixing, before first punch, after first punch, after last punch, after oven spring, and after baking was completed. Baked bread was cut in half and sampled at both the top center and bottom center of the loaf.

Samples were fixed in 0.1*M* phosphate-buffered (7) 4% glutaraldehyde at pH 6.7 for 3 hr, washed 3 times in buffer, postfixed in phosphate-buffered (0.05*M*, pH 6.7) 1% osmium tetroxide for 2 hr, rinsed 3 times in distilled water, and stained for 24 hr in 0.5% aqueous uranyl acetate at 4° C. Samples were dehydrated in a graded acetone series and embedded in Spurr resin (8).

Sections, 1 μm thick, and silver-gold thin sections were cut with glass and diamond knives on a Sorval MT-2b ultramicrotome. Plastic sections, 1-μm thick, were stained with Paragon stain (Paragon C. & C., Inc.), which is a mixture of *o*-toluidine blue and basic fuchsin; the mixture stains starch pink and protein blue. The sections were photographed with a Zeiss photomicroscope. Thin sections, which were first stained in 2% aqueous uranyl acetate and then in lead citrate, were viewed in a Philips EM 201 electron microscope at 60 kV.

RESULTS

Flour-Water Doughs

Effects of mixing on dough structure: General dough structure. The starch, in the form of individual granules, occurred in two distinct sizes—large (greater

[5]Mixing times of water-flour doughs were substantially lower than those for complete doughs (Table I) made from the same flours.

TABLE I
Description and Characterization of Hard Red Winter Wheat Flours

Flour	Protein (%)	Ash (%)	Mixing Time (min)	Water Absorption (%)	Loaf Volume (cc)
RBS-74 (composite)	12.4	0.42	3-3/4	63.1	1,003
RBS-75 (composite)	12.4	0.42	4-1/8	64.7	973
CI 12995	12.1	0.35	7	61.5	940
KS 501099	13.8	0.48	3/4	64.7	510

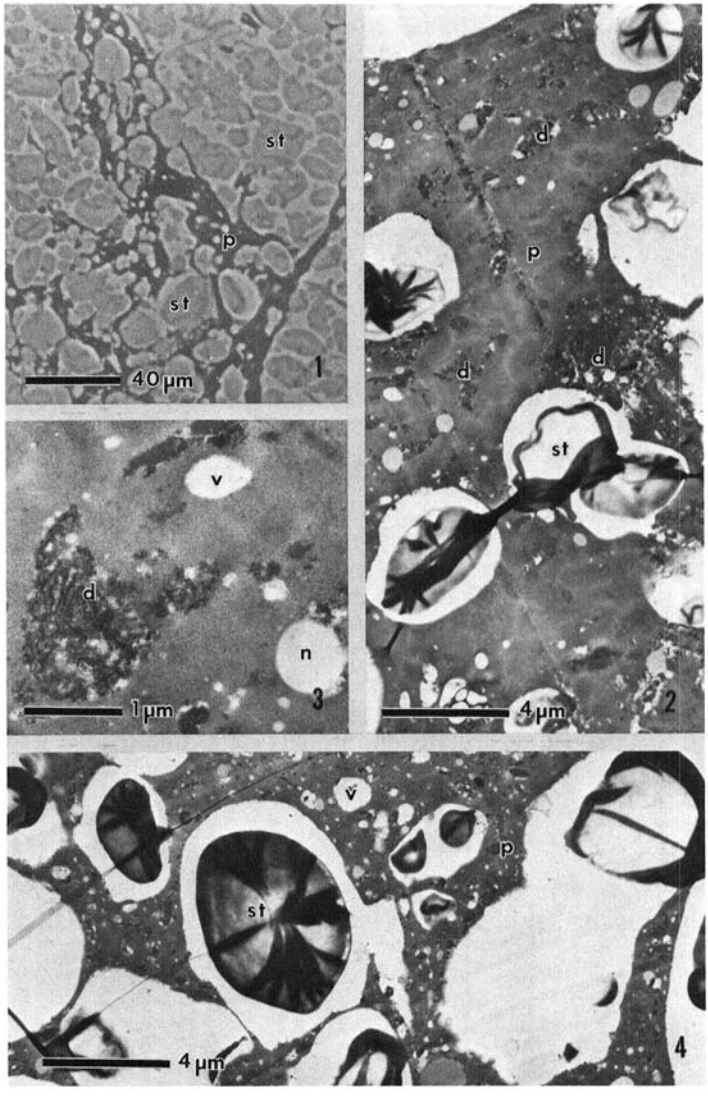

Fig. 1. LM of undermixed RBS-74 water-flour dough showing large bulky protein strands (p) and clumping of starch granules (st). **Fig. 2.** Undermixed RBS-74 dough with starch granules (st) embedded in protein strand (p). Note clumping of remnants (d) and lack of vacuoles. **Fig. 3.** Highly magnified undermixed dough showing small vacuoles (v), large area of remnants (d), and a flour lipid droplet (n). **Fig. 4.** Optimally mixed RBS-74 dough. Note well-developed fine protein strand (p) around starch granules (st). Vacuoles (v) are present and evenly distributed.

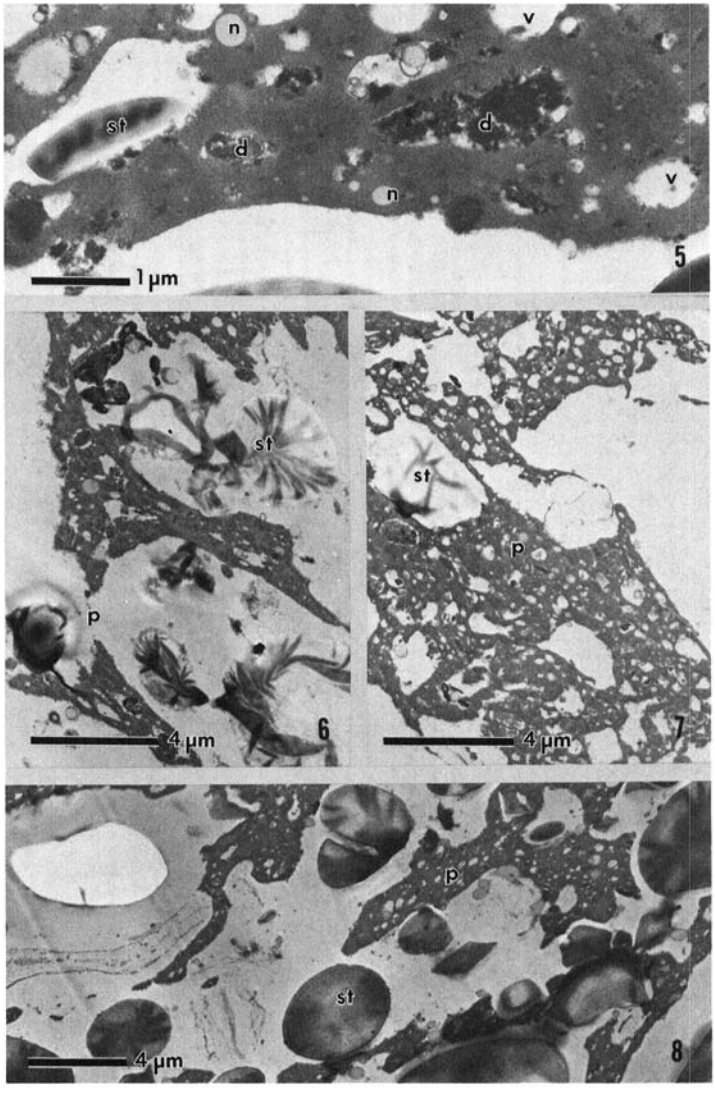

Fig. 5. Highly magnified optimally mixed dough with evenly distributed remnants (d), flour lipids (n), and vacuoles (v) in protein strand. Starch granule (st). **Fig. 6**. Overmixed RBS-74 dough showing rough appearance and apparently broken protein strand (p). Starch granule (st). **Fig. 7**. Overmixed RBS-74 dough showing highly vacuolated protein (p) around starch granule (st). **Fig. 8**. Overmixed CI 12995 dough containing broken protein strands (p) between starch granules (st) but fewer vacuoles than in overmixed RBS-74 dough (cf. Figs. 6 and 7).

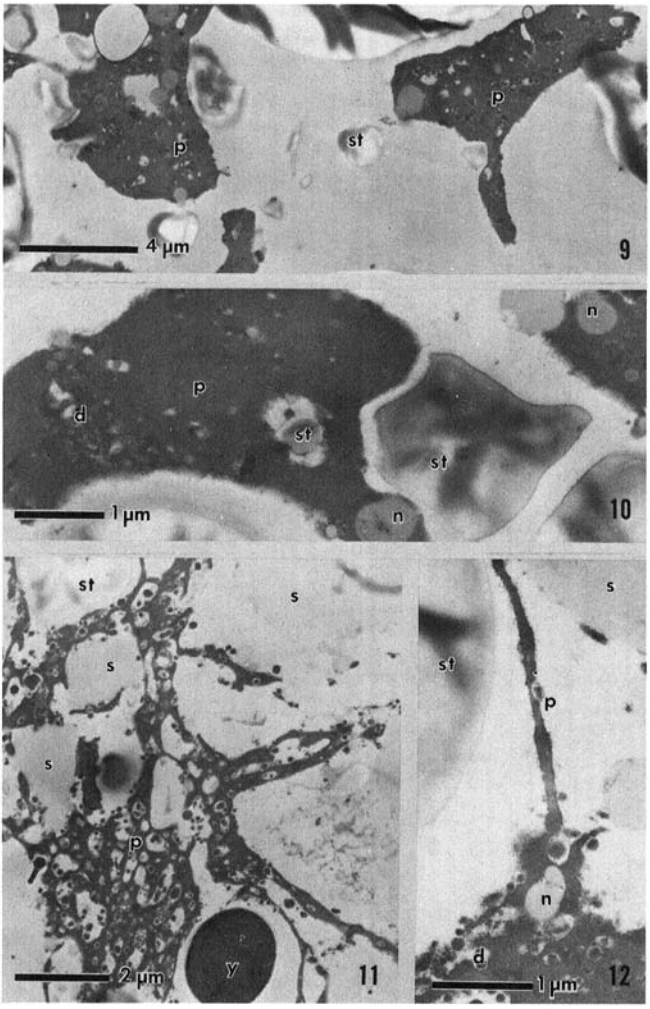

Fig. 9. Undermixed KS 501099 dough had protein (p) in clumps with evenly distributed remnants. Starch granule (st). **Fig. 10.** Optimally mixed KS 501099 dough was similar to undermixed dough. Protein (p) was in clumps, lacked vacuoles, and contained remnants (d) and flour lipids (n). Starch granule (st). **Fig. 11.** Optimally mixed complete dough from RBS-75 flour shows yeast cell (y), highly vacuolated protein strand (p) surrounding shortening (s), and starch granule (st). Note that remnants are in vacuoles. **Fig. 12.** Highly magnified complete dough showing fine protein strand (p) between starch granule (st) and shortening lipid droplet (s). Remnants are located in small vacuoles (d). Note that native lipid (n) stains similar to shortening.

than 20 μm) and small (smaller than 10 μm). The protein component was in the form of sheets and fibrils that contained numerous inclusions, three types of which were identified—1) small voids within the protein, termed vacuoles, 2) spherical electron-lucent bodies, tentatively termed lipid droplets for reasons to be discussed later, and 3) electron-dense, irregularly shaped structures (thought to be remnants of endosperm organelles and membrane system), termed remnants.

RBS-74. The undermixed RBS-74 dough was characterized by many groupings of large and small starch granules separated from the protein (Fig. 1). Small starch granules were usually associated with, or embedded in, the protein matrix (Fig. 2). The protein was composed of large, bulky strands that contained many inclusions (Figs. 2, 3). Few vacuoles were present in the protein strands, and neither the electron-dense inclusions nor the droplets of flour lipid were evenly distributed. In overall appearance, the constituents of the dough had not been uniformly integrated.

In the optimally mixed RBS-74 dough, nearly every starch granule was surrounded by thin protein strands and sheets (Fig. 4). The electron-dense remnants, which were present within some of the many vacuoles, and droplets of flour lipid were scattered throughout the protein strands (Fig. 5). In overall appearance, constituents were evenly distributed.

The grossly overmixed RBS-74 dough was characterized by many broken protein strands and vacuoles (Figs. 6,7). The broken strands and vacuoles imparted a rough appearance to the protein. In many strands, vacuoles opened to the outside, almost bisecting the protein. Starch granules were in small groups no longer surrounded individually by protein. Inclusions in the protein were evenly distributed. Many, however, were present outside the strands. The overmixed doughs appeared highly disrupted.

CI 12995 strong flour. Both the undermixed and optimally mixed doughs of CI 12995 seemed identical to corresponding doughs of RBS-74. Although similar to the overmixed RBS-74, the overmixed CI 12995 dough seemed less damaged by excessive mixing, reflecting perhaps the good mixing tolerance of the CI 12995 strong, long-mixing flour. In overmixed doughs, fewer and smaller vacuoles, fewer broken strands, and smoother-appearing protein strands occurred in CI 12995 than in RBS-74 (compare Fig. 8 with Figs. 6 and 7). Overmixed doughs of CI 12995 had starch granules that remained surrounded by protein and few electron-dense inclusions exterior to the protein.

KS 501099 weak flour. The undermixed KS 501099 dough primarily consisted of starch granules partially surrounded by protein fragments (Fig. 9). The protein fragments contained uniformly distributed inclusions (remnants and lipid droplets).

The optimally mixed KS 501099 dough was similar to the undermixed dough in that it lacked vacuoles (Fig. 10). Although the development of protein strands was evident, most strands were short and broken. The overmixed dough appeared to be similar to the optimally mixed dough, but had more broken strands and few vacuoles and appeared to be highly disrupted.

Complete Dough

Optimally mixed dough (RBS-75 flour). Several major constituents, starch, protein, yeast cells, and large lipid droplets (presumably from the added short-

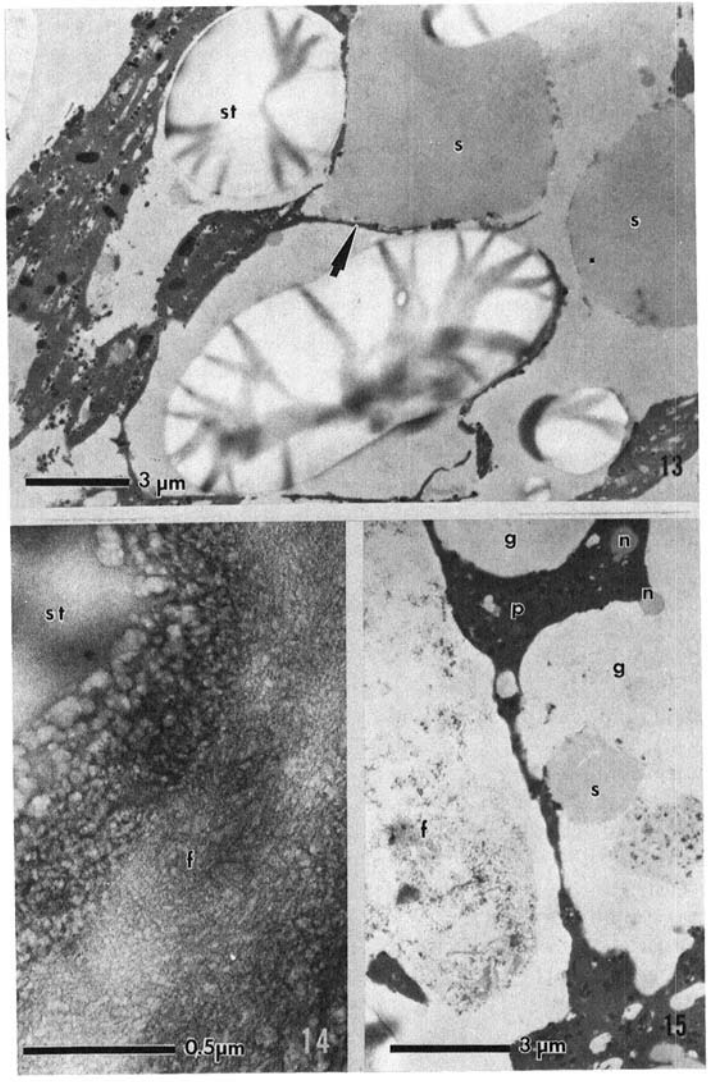

Fig. 13. Complete dough after first punch. Note fine protein strands (arrow), starch granule (st), and shortening (s). Also note that number of vacuoles has decreased compared with optimally mixed dough (Fig. 11). **Fig. 14.** Gelatinized starch (f) bottom center of loaf after only proof appears fibrous and is gelatinized first at center (f) of starch granule rather than at edge (st = starch ungelatinized at edge). **Fig. 15.** Bottom center of loaf after oven proof. Note lack of vacuoles in protein (p) and no change in either native lipid (n) or shortening (s). Starch is gelatinized and appears fibrous (f). Gas vacuoles (g).

ening), could be identified in the dough (Fig. 11). Many starch granules were surrounded by thin protein sheets and strands (Fig. 12). The protein contained many uniformly dispersed, electron-dense remnants in vacuoles (Fig. 11). Yeast cells stained densely and were evenly distributed throughout the dough. Large lipid droplets, which were formed from the added shortening (they were absent in water-flour dough), were also scattered in the dough and did not seem consistently associated with either protein or starch.

Fermentation. Doughs sampled before the first punch seemed similar to the mixed dough, except gas vacuoles had started to form because of the action of yeast. Punched doughs were similar to freshly mixed and partially fermented doughs (before first punch) (Fig. 13). Gas vacuoles, were not present, and the edges of protein strands were smoother than they were in mixed doughs.

Top center of loaf after oven spring. After oven spring (5 min of a 24-min bake) loaves were cut in half and sampled from the crumb-dough transition zone. Starch granules had not become gelatinized. Protein strands were thin and had smaller vacuoles and smoother edges than did protein strands from previous stages. The shortening was not consistently associated with any structures.

Bottom center of loaf after oven spring. Starch granules from the bottom center of the loaf varied widely in degree of gelatinization after oven spring. Starch had started to gelatinize from the interior of the granule and appeared fibrous and greatly dispersed (Fig. 14). The protein, which was in the form of thin strands, had smooth edges and a few small vacuoles. Large lipid droplets were

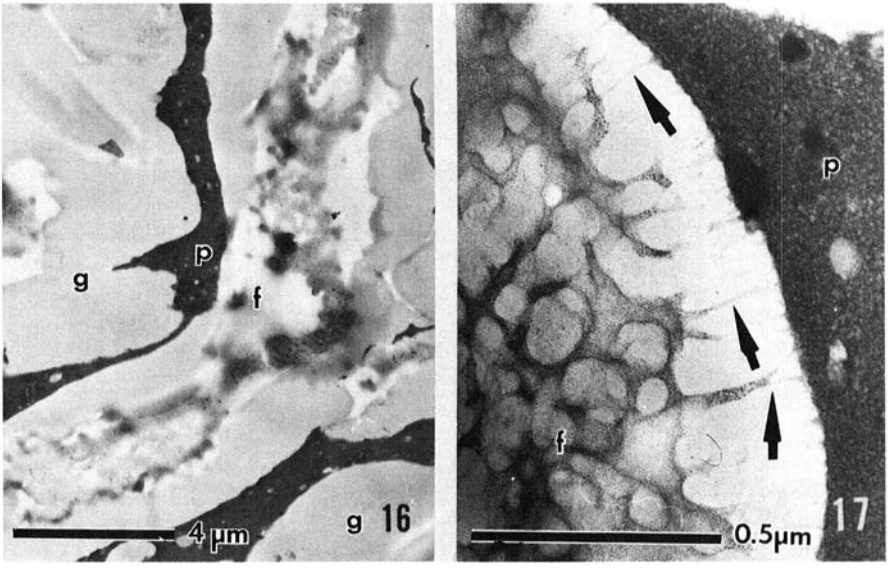

Fig. 16. Top center of loaf immediately after baking. Note gelatinized starch (f) between thin protein strands. Note lack of vacuoles in protein (p). Gas vacuoles (g). **Fig. 17.** Highly magnified protein (p)-gelatinized starch (f) interface. Note fine connections between starch and protein (arrows).

not associated consistently with any identifiable major structures or components (Fig. 15).

Top and bottom center of loaf after baking. Much starch from the sampled areas of the completely baked loaf, especially from the bottom, was gelatinized. The starch greatly expanded into long, bulky fibrous strands interwoven with the protein (Fig. 16). The gelatinized starch was linked to the protein by thin fibrils (Fig. 17). The protein was nonvacuolized, had smooth edges, and occurred as thin strands (Figs. 16,17). The shortening was not consistently associated with any structures.

DISCUSSION

The endosperm proteins of wheat have the unique property of forming gluten when wetted and mixed with water. Gluten imparts physical properties that differ from those of doughs made from any other cereal grain. It is gluten formation rather than any distinctive nutritive property that gives wheat its prominence in the diet. When water is added to wheat flour and mixed, the water-insoluble proteins hydrate and form gluten, a complex coherent mass in which starch, added yeast, and other dough components are embedded. Thus, in reality the gluten is the skeleton or framework of wheat-flour dough and is responsible for gas retention, which makes production of lightly leavened products possible.

Although the structure of wheat-flour doughs has been described (1,2), the effect of mixing on structure of doughs from good- and poor-quality flours has not. Our study provides such information.

An optimally mixed dough formed from a good breadmaking composite flour results in even and continuous distribution of protein around starch granules and even distribution of inclusions within the protein. In our study, the undermixed composite-flour dough failed to meet those conditions. In the overmixed dough, distribution of inclusions within the protein was even, but the protein was not continuous. Protein strands were broken and the protein contained many large vacuoles. The vacuoles apparently weakened the protein by producing localized thin protein strands that were disrupted easily. Those results agree with the findings of Baker and Mize (9), who measured (by changes in dough density) vacuole formation in doughs and effects of vacuole formation on bread quality. Doughs that are inadvertently overmixed can be relaxed and then remixed to form an optimally mixed dough (10). We found that when an overmixed dough was allowed to relax, the protein vacuoles decreased in size and number (unpublished data). Possibly the relaxation and gentle remixing "mends" the broken protein strands so that an optimally mixed dough is restored.

Although both flour lipids and shortening contribute to the size and overall quality of the bread, the mechanisms of that contribution differ (11). We were able to differentiate between wheat-flour lipids and shortening, but only the flour lipids were consistently associated with the protein. The shortening was not consistently associated with either protein or starch. Perhaps our fixation and dehydration technique extracted some lipids and disrupted the association, or perhaps the interaction is of a type not visible by electron microscopy.

The poor-quality flour dough differed strikingly from the good-quality flour dough. The protein strands of the poor flour dough broke easily, even before the protein inclusions were uniformly distributed. In good-quality flour, the tensile

strength of the hydrated proteins apparently governs the structure of the dough and the bread. That was quite apparent when a good-quality flour was grossly overmixed—the protein strands remained continuous, with relatively few being broken. This "stability" to overmixing (and even undermixing) is one of the most important and desirable characteristics of good-quality flours.

Our results point to the significance of starch and starch-protein interaction in the baked bread. They are in agreement with the findings of Yasunaga *et al.* (12) on factors that govern gelatinization of starch during baking, of Derby *et al.* (13) on the significance of limiting amounts of water in gelatinization of starch in baked or cooked products, and of Wehrli and Pomeranz (14) on the significance of starch-protein interactions in dough and bread.

Acknowledgment

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