Freeze-Fracture Ultrastructure of Wheat Flour Ingredients, Dough, and Bread[1]

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ABSTRACT

The structures of isolated flour components of mixed doughs (containing several combinations of ingredients), of fermented doughs, and of bread crumb were examined by the freeze-fracture technique. Although the shapes of the small and large starch granules were unaltered in doughs, the gluten and the water-soluble structures appeared completely different in the complex-dough system. In general, water was distributed in three forms: 1) coating around starch granules and yeast cells, 2) droplets, and 3) large areas; all three changed with protein development. Protein development was followed from a protein network in a flour-water dough to a sheetlike protein in a complete dough (containing flour, water, yeast, sugar, salt, shortening, malt, and oxidant). Both compositional and physical (dough development) effects were indicated. A transition stage between the two structures appeared after sugar was added. Fermenting a flour-water-yeast-salt dough did not affect the protein network structure, but fermenting a complete dough altered the sheetlike protein to a fine network. In bread, regular dense-structured sheets were observed. In most doughs, protein-starch interaction was clearly visible; thin "pearl chains" or thin protein strands connected starch and protein. Those interactions intensified after fermentation. In bread crumb, protein and starch were tightly connected.

Breadmaking involves complex, multiple interactions of wheat flour components. Such interactions can be followed by physical, chemical, and microscopic methods. Microscopy is a particularly useful and powerful instrument for studying the ultrastructure and functional relationships of the interactions in situ. Several workers, ie, Evans et al (1977), Bechtel et al (1978), and Chabot et al (1979), have recently used transmission electron microscopy (TEM) and scanning electron microscopy (SEM) in flour, dough, and bread studies.

Bechtel et al (1978) concluded, on the basis of TEM studies, that protein strands provide a matrix network in a mixed dough and that in baked bread most of the starch is gelatinized into fibrous strands interwoven with thin protein strands. The SEM studies of a water-flour dough by Evans et al (1977) showed a thin gluten sheet with a crepelike texture and small random pockets. According to SEM pictures by Chabot et al (1979), unfixed bread has air cells coated with a relatively smooth, thin, continuous protein layer with embedded starch granules.

The structure of commercial gluten was studied by TEM and SEM (Cumming and Tung 1975), gluten components by TEM (Crozet et al 1974), and gluten and glutenin by SEM (Orth et al 1973a, 1973b; Tu and Tsen 1978). Extensive studies on the structure of soybeans and soy products were conducted by Wolf and Baker (1975).

Dehydrating or freeze-drying specimens during preparation for SEM may produce artifacts or mask surface details (Allen et al 1977). Moreover, exposure to buffers, fixatives, and dehydrating agents before drying may alter the protein matrix and liberate starch granules from the matrix (Chabot et al 1979). To examine the relationship between the structures of starch and of baked goods, the components of the system should be practically undisturbed, and the best treatment is no treatment (Chabot 1979).

For those reasons and because water, next to starch, is the main quantitative ingredient of dough and bread, dough and bread should be studied with minimal, or preferably without, chemical fixation and dehydration. The freeze-fracture technique, therefore, is a promising method to investigate water distribution in dough and bread. In the freeze-fracture technique, a replica of the sample is made by rapid freezing, fracturing, and finally shadowing with platinum. The cleaned replicas of the frozen specimens can be evaluated with high magnification and resolution in an electron microscope.

The freeze-fracture technique involves making a platinum-carbon replica of a fracture plane through a frozen sample. Hall (1950) initially suggested the technique and made an apparatus in which the surface of ice could be sublimed to create a relief view of hydrated silver halide specimens. Meryman (1950) carried this procedure one step further by fracturing the frozen specimen before replication. A refined version of the technique used material fractured in vacuo and then sublimed (Meryman and Kafig 1955). Steere (1957), however, was the first to produce freeze-etch replicas of biological material. Since then, several advances in instrumentation and techniques have been developed (Koehler 1972). Antognelli (1980) used freeze-fracturing to study the structure of pasta products. Freeze-etch studies were performed on starches by Mühlethaler (1965), Leonard and Sterling (1972), Hölzl (1973), Chabot et al (1978), and Allen et al (1977).

We know of no freeze-fracture studies on the ultrastructure of dough and bread. Even though the freeze-fracture method overcomes artifacts resulting from fixation and dehydration, artifacts still may occur during freezing, fracturing, and shadowing. Contamination and heat damage must be minimized, and adequate contrasting platinum shadowing must be used to demonstrate details of the fine structure of biological systems.

The purposes of this freeze-fracture investigation were 1) to study the ultrastructure of dough ingredients, 2) to follow structural interactions of the dough components in dough and bread, and 3) to evaluate changes in water distribution and overall structure in doughs containing several combinations of components and in fermented doughs and bread crumb.

MATERIALS AND METHODS

Dough Ingredients

Starch, gluten, and water solubles were isolated according to Finney (1971) from a regional baking standard flour—an untreated, experimentally milled composite flour of several hard red winter wheat varieties grown at several locations throughout the Great Plains in 1976.

The fractions, commercial malt, and soy flour were prepared for freeze-fracturing. Starch (moisture, 3.6%; protein, 1.0%, dry basis) (1.0 g) was suspended in 1.3 ml of water. Gluten (moisture, 2.8%; protein, 81.0%, dry basis) was kneaded with excess water. Water solubles (moisture, 4.3%; protein, 19.9%, dry basis) were mixed with a few drops of water to give a thick slurry. Malted barley flour (Amylomalt, Ross Industries, Inc., Wichita, KS) (500 mg) was suspended in 20 drops of water. Soy flour (Baker's Nutrisoy, Archer Daniels Midland Co., Minneapolis, MN) (500 mg) was suspended in 1.2 ml of water.

Doughs and Bread

Doughs and bread were prepared from a regional baking

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standard 1978 flour. Some of the flour's characteristics (at 14% mb) were: protein, 12.3%; ash, 0.42%; mixing time, 4¼ min; water absorption, 64.0%; and loaf volume, 970 cc (per 100 g of flour).

Doughs were prepared from 10.0 g of flour and bread from 100.0 g of flour according to the procedure described by Bruinsma and Finney (1981). For combinations of ingredients (in the series from flour-water to complete dough), the ingredients (compressed yeast, 5.3 ± 0.2 g; salt, 1.5 g; shortening, 3.0 g; sugar, 6.0 g; malt, 0.25 g [52 dextrinizing units per gram]; ascorbic acid, 50 ppm) were added per 100 g of flour. In wheat flour-soy flour combinations, 10% of the wheat flour was replaced by soy flour.

Freeze-Fracture

For freeze-fracture, a modified Denton DFE-3 instrument operated on a Denton DV-502 vacuum evaporator was used. Single components (slurries of starch, water solubles, malt flour, and soy flour), gluten, doughs after mixing or fermentation, and bread crumb from the center of the loaf were mounted in gold specimen holders and snap-frozen immediately in monochlorodifluoromethane cooled with liquid nitrogen. Samples were transferred to the freeze-fracture instrument, defrosted at −100° C for about 10 min, and fractured and shadowed at −150° C and 7×10^{-6} torr. Samples were generally not etched, because etching destroyed fine structure. Etching, when conducted, was done before shadowing at −100° C for 30–60 sec. Etching was used to determine where the water (ice) was located. Areas that were etched indicated that water, in the form of ice, was present. By comparing etched and nonetched replicas, we were able to show that both the large water areas and the water droplets were in reality ice crystals. A shroud cooled by liquid nitrogen surrounded the specimen during processing. The replicas were floated off the specimen holders with 50% (v/v) sulfuric acid (5–20 hr), cleaned in 50% (v/v) chromic acid (2–20 hr), and washed three times in double-distilled water. The replicas were picked up on formvar-coated grids and examined in a Philips EM-201 electron microscope operated at 60 kV. The microscope negatives were contacted on another sheet of film to form an internegative, which then printed to form positive prints with dark platinum shadows.

RESULTS

Dough Ingredients

Starch. Most starch granules were crossfractured and exhibited a granular ultrastructure with sharp edges (Fig. 1). Granules appeared in two distinct shapes (not shown). Large granules (type A) were egg-shaped with a long diameter of about 16–20 μm and a short diameter of about 10–12 μm. The crossfracture was oval or round. The small granules (type B) were spherical and had diameters of about 2–6 μm. Longitudinal fractures of the large granules showed a wrinkle in the middle. The starch granules were completely surrounded by water. The term "water" refers to water in the frozen state, ie, ice crystals formed during replication. Water in the various components was demonstrated by etching the samples for 1 min at −100° C before replication (figures not shown).

Gluten. The freeze-fractured gluten surface appeared as a large sheetlike matrix. Within the sheet, a layerlike pattern arrangement was visible. Numerous relatively small inclusions of different sizes were present. They had smooth surfaces and were droplet-shaped, which might suggest their lipid character. No water was detectable (Fig. 2).

Water Solubles. The water solubles are a mixture of carbohydrates (including simple sugars and pentosans), proteins (including amino acids and peptides), minerals, and other components. The uneven fracture surface consisted of rounded bumps irregularly segmented by bright, depressed strands. Irregularly formed (or shapeless) particles were within the lumpy surface. The structure showed no distinctly (or sharply) shaped elements because the components were highly hydrated (Fig. 3).

Malted Barley Flour. Structures of the large and small starch granules were similar to those of the granules in the wheat flour. Most starch granules appeared intact (no data given). Figure 4 shows part of a small starch granule, partly digested from the outside. The digestion is either in a broad area (upper right) or more limited (bottom left). Long digested furrows were observed in large starch granules (Fig. 5).

Soy Flour. Two structural elements were observed in the freeze-fracture micrographs of the processed soy flour: 1) densely granulated cell-wall material (Fig. 6) and 2) partly disrupted protein bodies, consisting of a protein network of narrow protein strands, partly surrounded by denser protein material (Fig. 7).

Mixed Doughs

Flour-Water. The flour-water doughs (Figs. 8 and 9) were composed of the two types of starch granules described above, a coarse protein network, and large water areas. Starch granules were surrounded by a layer of water (Fig. 9). The protein network, distributed between the starch granules, enclosed water droplets (Figs. 8 and 9). The protein structure differed significantly from the structure of isolated gluten. No sheetlike protein and no lipid-like inclusions were visible in the dough. The large water areas appeared like "dry clay soil" with a fine webbed type of structure (Fig. 8), and their structure was similar to that of the water-soluble fraction. The webbed type of structure is not sublimed during etching and may represent minor concentrations of various solutes.

Flour-Water-Yeast. Adding yeast altered the structure little; the yeast cells were about the size of the small starch granules. (No figure is given.)

Flour-Water-Soy. Adding soy flour somewhat altered the water distribution in the protein network (Fig. 10). The water droplets were larger than those in the flour-water dough and often connected; the protein strands appeared stretched. Protein bodies in the soy flour were visible (Fig. 11). The other structural elements, cell walls and lipid droplets, were not detected.

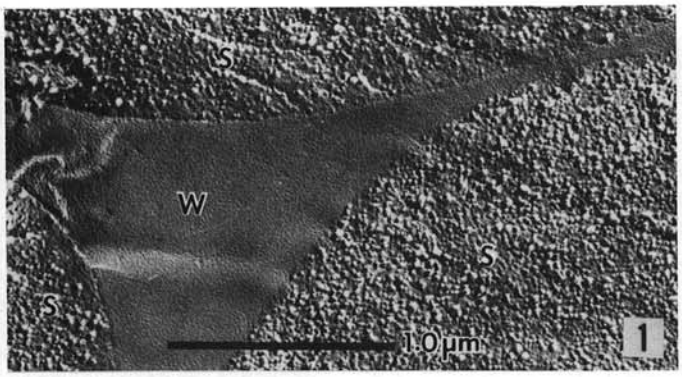
Flour-Water-Yeast-Salt. The large water area was altered after salt addition in that it contained "pearl chains" (Fig. 12). The webbed type of structure in the flour-water dough (Fig. 8) was replaced by fine pearl chains, which sometimes interacted with the starch granules (Fig. 13). Yeast cells were crossfractured or visible from the outside and surrounded by a water coat, which contained some material adhering to the yeast cell membrane (Fig. 12, left side).

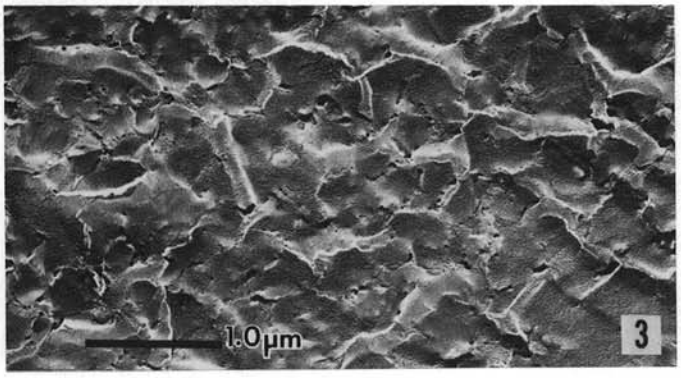
Flour-Water-Yeast-Salt-Shortening. Most structural components (Fig. 14) were similar to those in the previously described doughs. When shortening was added, two kinds of lipid particles appeared: 1) layered ones, irregularly shaped but having sharp edges and dense dotted surfaces (Fig. 15), and 2) large layered balls, the largest the size of the smallest starch granules. The internal layers were dense-dotted. The shortening balls were surrounded by water (Figs. 16 and 17).

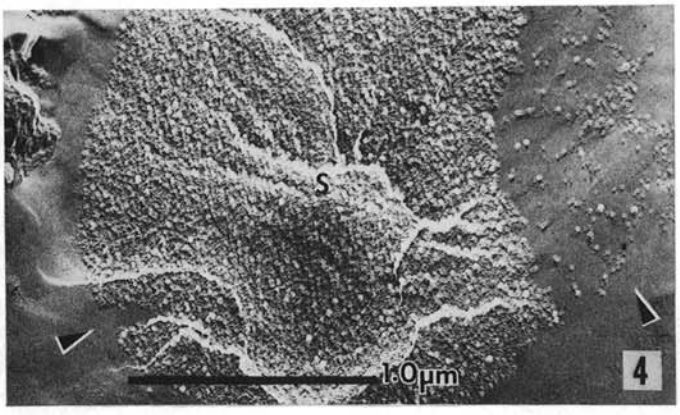
Flour-Water-Yeast-Salt-Shortening-Sugar. Adding sugar dramatically changed water distribution and protein appearance (Figs. 18 and 19). The water coats surrounding the starch granules were narrowed. The water droplets were smaller and less clearly discernible, and water seemed to be distributed better in the protein matrix than in doughs containing no added sugar. The protein seemed to be in a transition stage between network and sheetlike structures. The water formed no droplets, as in the simpler doughs, but was diffusely distributed. The protein did not appear sheetlike as in the complete dough. Lipid inclusions in the protein (Fig. 19) were similar to the inclusions in the isolated gluten (Fig. 2). The lipid inclusions were small, spherical, and often surrounded by water. Some protein-starch interaction is shown (Fig. 19).

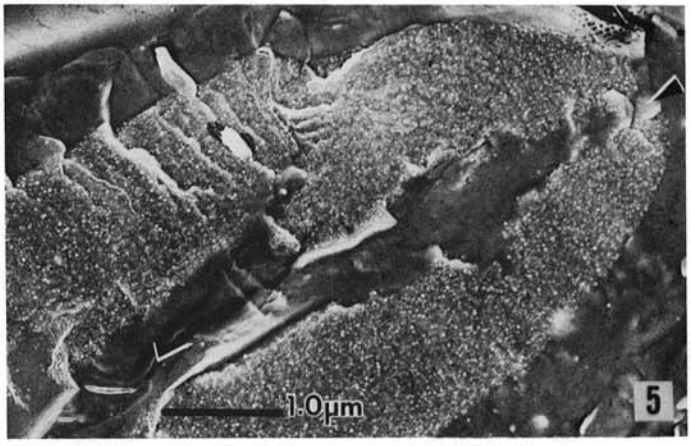
Flour-Water-Yeast-Salt-Shortening-Sugar-Malt. Adding malt resulted in no significant structural changes (no data given).

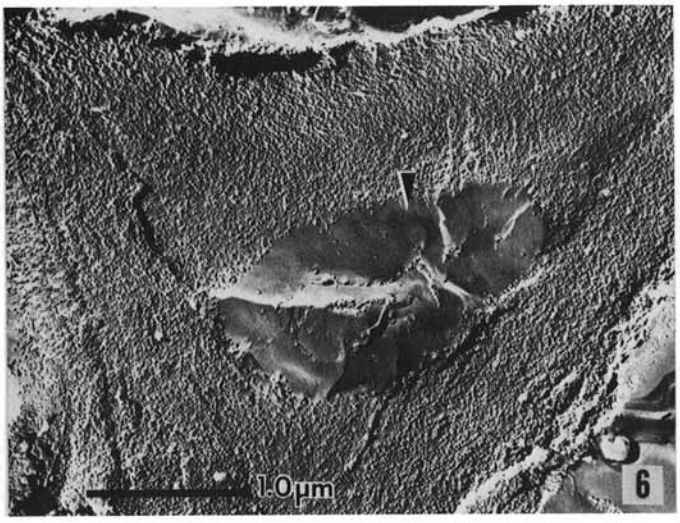
Complete. In complete dough, both kinds of shortening particles were present (Fig. 20). The water interphase between protein and starch is shown in Fig. 21; no obvious protein-starch interaction was visible in the extremely thin water coat, but some small lipid inclusions were observed. Large smooth-water areas filled some spaces between the starch granules (Fig. 22). With an oxidant (ascorbic acid) added, the protein structure was further developed and generally appeared as large sheetlike areas with numerous small lipid inclusions (Fig. 23). Crossfractured yeast cells (Fig. 23) exhibited several organelles, eg, a nucleus with pores on both faces











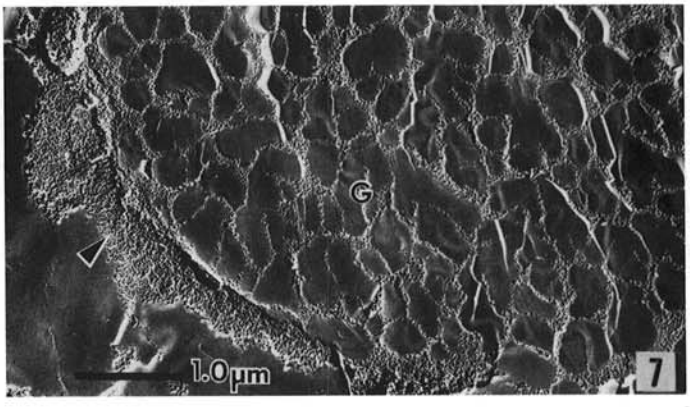


Fig. 1. Small portions of three cross-fractured starch granules **(S)** (isolated) from wheat flour surrounded by water **(W)** (ice). **Fig. 2.** Isolated gluten showing a layerlike pattern in a matrix sheet with lipid particles (arrows). **Fig. 3.** Water solubles, showing an uneven fracture surface. **Fig. 4.** Part of a small starch granule from malted barley flour, partly digested (arrows). **Fig. 5.** Part of a large starch granule from malted barley flour with large digested furrow (arrows). **Fig. 6.** Cell wall material from soy flour with intercellular space (arrow). **Fig. 7.** Protein granule **(G)** from defatted soy flour surrounded by adherent material (arrow).

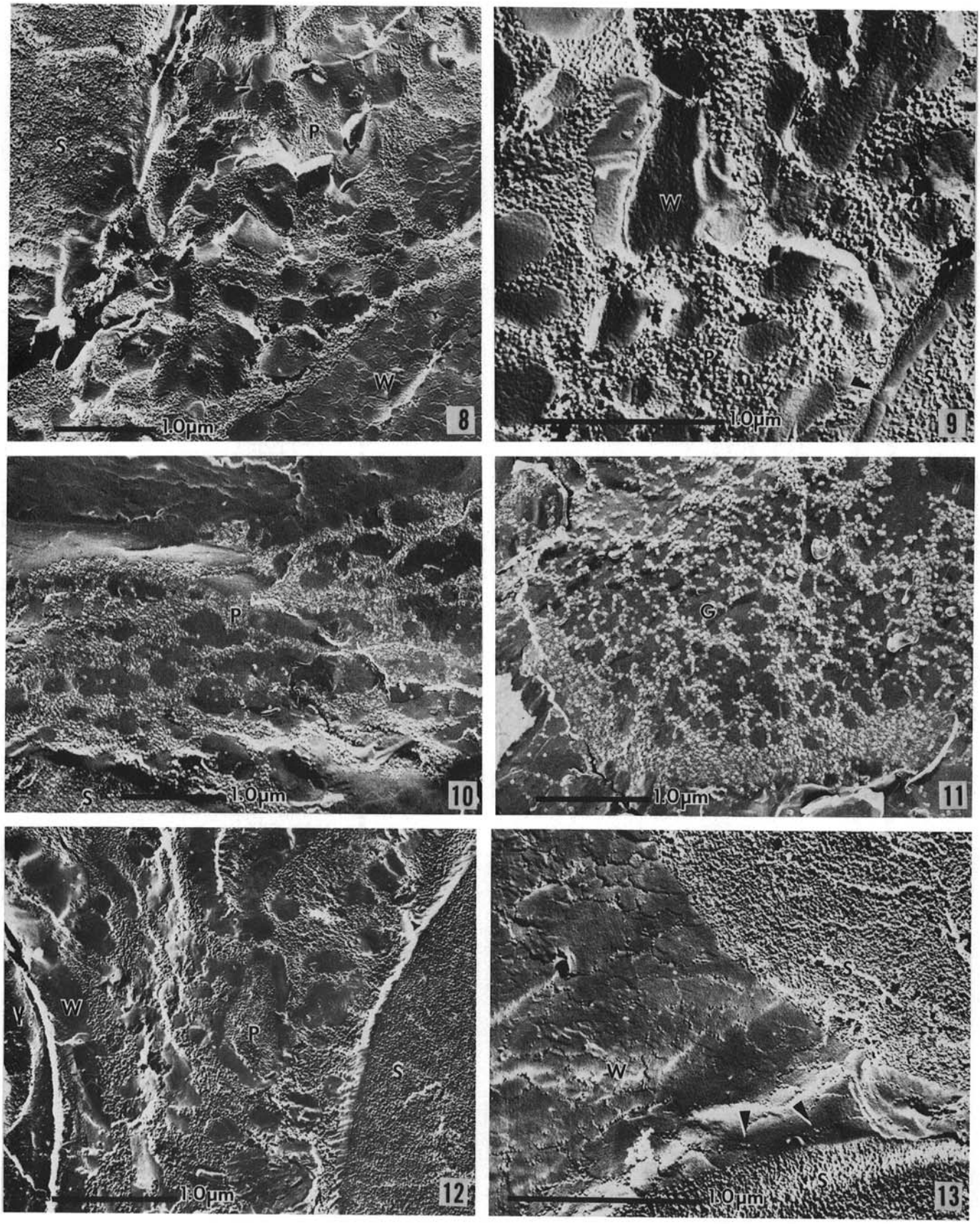
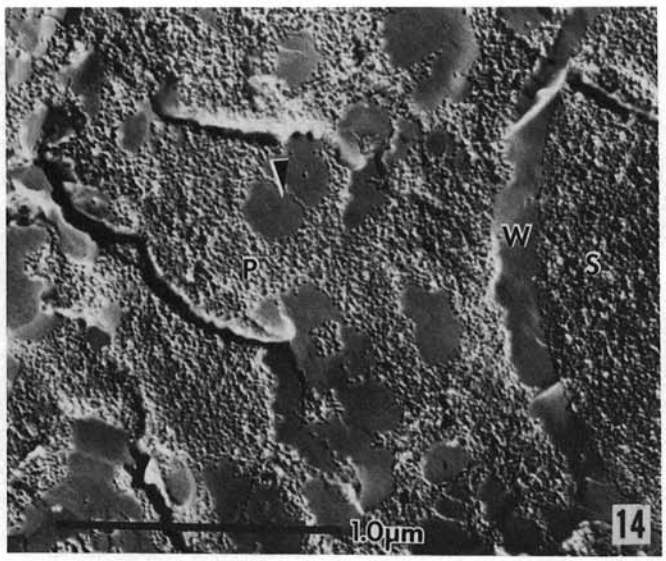
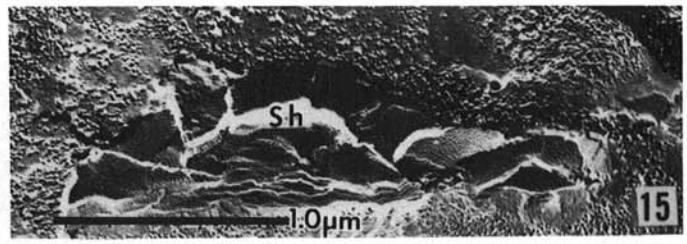
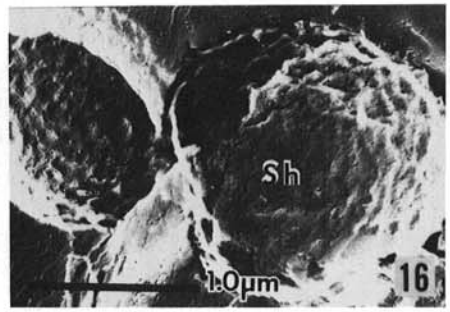
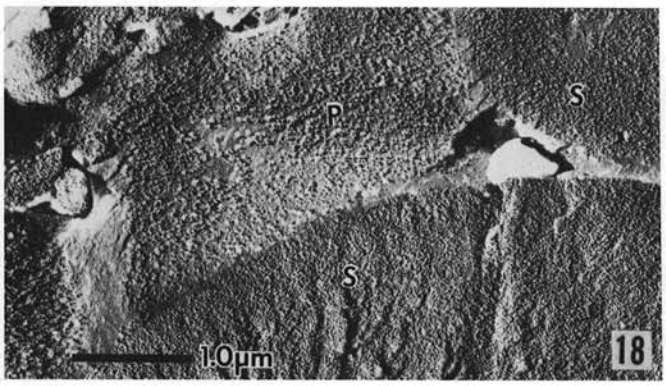


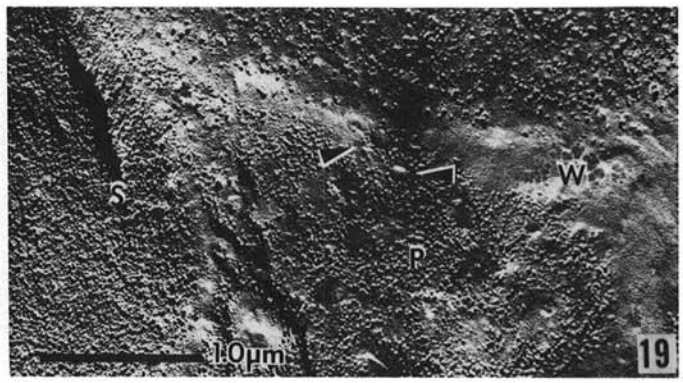
Fig. 8. Flour-water dough with starch granule **(S)**, protein network **(P)**, and large water area **(W)**. **Fig. 9.** Flour-water dough at higher magnification, showing starch granule **(S)** with water coat (arrow), water droplet **(W)**, and protein **(P)**. **Fig. 10.** Flour-water-soy dough with starch granule **(S)** and protein network **(P)**. **Fig. 11.** Flour-water-soy dough with soy protein granule **(G)**. **Fig. 12.** Flour-water-yeast-salt dough with starch granule **(S)** and protein network **(P)**. Note the smooth surface of water droplets and yeast cell **(Y)** with water coat **(W)**. **Fig. 13.** Flour-water-yeast-salt dough, showing starch granule **(S)** in large water area **(W)** with "pearl chains" that interact with the starch granules (arrows).

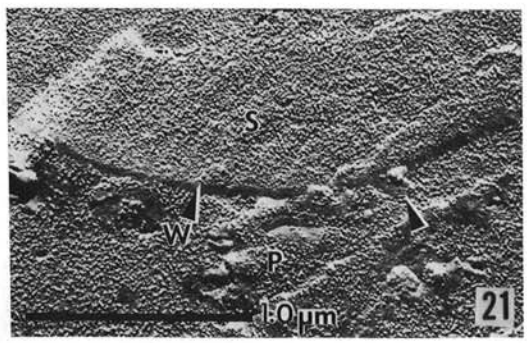












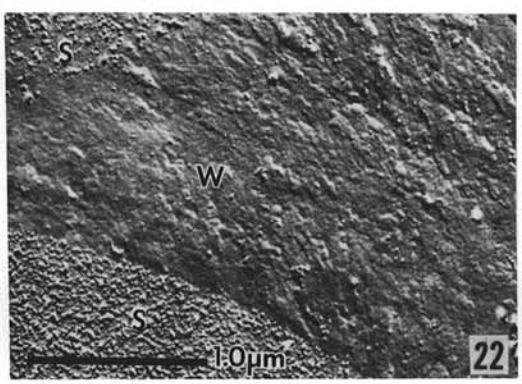


Fig. 14. Flour-water-yeast-salt-shortening dough, showing starch granule **(S)** with protein network **(P).** Note water coat **(W)** around the starch granule and water droplets (arrow). **Fig. 15.** Layered shortening **(Sh)** in flour-water-yeast-salt-shortening dough. **Figs. 16** and **17.** Large layered shortening balls **(Sh)** in flour-water-yeast-salt-shortening dough. **Fig. 18**. Flour-water-yeast-salt-shortening-sugar dough, showing sheetlike protein **(P)** between starch granules **(S). Fig. 19**. Flour-water-yeast-salt-shortening-sugar dough, showing small portion of a starch granule **(S)** and protein **(P)** with large water area **(W)** and lipid inclusions (arrows). **Fig. 20**. Complete dough, showing sheetlike protein **(P)** with shapeless shortening **(Sh)** and lipid particles (arrows). **Fig. 21.** Complete dough, showing part of starch granule **(S)** with thin water coat **(W)** and lipid inclusions (arrow) in the protein matrix **(P). Fig. 22.** Complete dough, showing parts of starch granules **(S)** with large water area **(W). Fig. 23.** Complete dough with yeast cell **(Y)**, part of starch granule **(S)**, protein sheet **(P)**, and lipid inclusions (arrows).

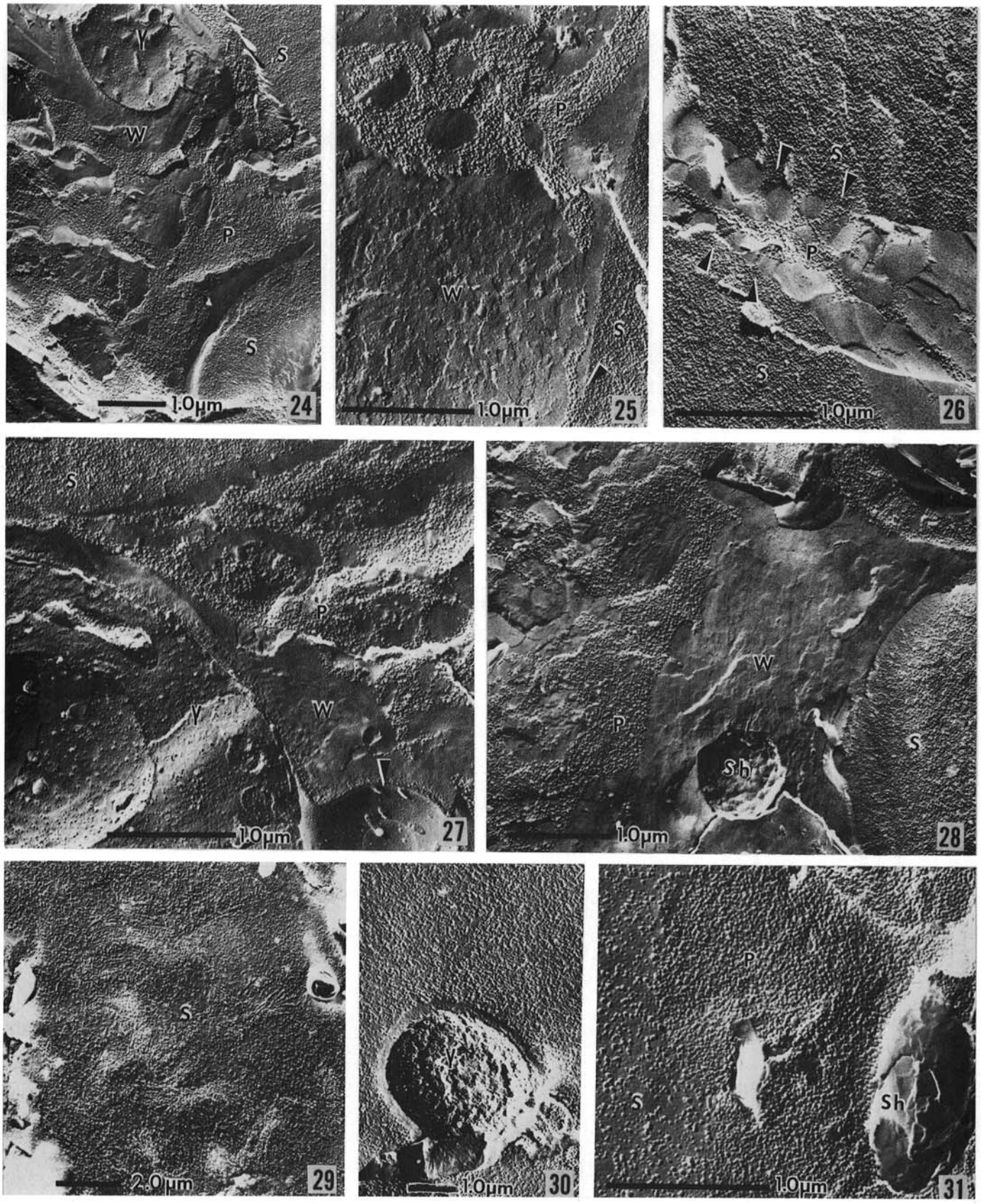


Fig. 24. Fermented flour-water-yeast-salt dough with parts of starch granules **(S),** yeast cell (showing the cell membrane), and protein network **(P)** surrounded by water **(W). Fig. 25.** Fermented flour-water-yeast-salt dough, showing starch granule **(S)** with long protein strand **(P).** Note protein-starch interaction (arrow) and large water area **(W). Fig. 26.** Fermented flour-water-yeast-salt dough, showing intensive starch **(S)**-protein **(P)** interaction (arrows). **Fig. 27.** Fermented complete dough with budding (arrow) from a yeast cell **(Y)** starch granule **(S),** protein network **(P),** and water **(W). Fig. 28.** Fermented complete dough showing a shortening ball **(Sh)** in a large water area **(W),** protein network **(P),** and a starch granule **(S). Fig. 29.** Bread crumb, showing gelatinized starch **(S)** with fibrous pattern. **Fig. 30.** Bread crumb, showing a "killed" yeast cell **(Y)** still surrounded by a water layer. **Fig. 31.** Bread crumb showing shortening **(Sh)** in dense protein **(P)** tightly connected to starch **(S).**

of the membrane, mitochondria, Golgi bodies, and a few lipid droplets.

Complete Dough with Soy. Adding soy flour resulted in no significant changes; soy protein bodies were not detected (no data given).

Fermented Doughs

Flour-Water-Yeast-Salt. Figure 24 shows an overview: parts of starch granules, protein network, distributed water, and a small yeast cell. The fermented dough was different in two ways from the mixed flour-water-yeast-salt dough. In addition to the regular protein network, it had long protein strands (Fig. 25), developed during fermentation. Also, its protein-starch interaction was considerably intensified (Fig. 26). No gas vacuoles produced by the yeast could be detected, probably due to specimen handling, which involved dough compaction.

Complete. Higher yeast cell activity was clearly visible in the form of greater yeast-cell concentration and dividing (budding) yeast cells (Fig. 27). Fermentation of a complete dough resulted in many structural changes: the sheetlike protein structure of the mixed dough was replaced by a protein network; the water droplets (Fig. 28) appeared similar to those in the fermented flour-water-yeast-salt dough and the mixed incomplete doughs; and the water coat around the starch granules was broadened. The large smooth-water areas were still comparable to those in the mixed complete dough.

Bread Crumb

Baking altered all structures substantially: the starch was gelatinized, so the starch granule shape could hardly be seen (Fig. 29), and fibrous structures appeared, probably partially from starch retrogradation. The protein was converted to even sheets of dense particles with small inclusions. Yeast cells were "killed" and shrunken (Fig. 30), thus appearing somewhat covered by denatured proteins, but were still surrounded by a water coat. The granular ultrastructure of the protein was denser than that of the starch (Fig. 31). This interpretation is based on the fact that during baking the protein condenses whereas the starch gelatinizes and expands (Bechtel et al 1978). Therefore, the protein matrix will have a denser pattern of particles than the dispersed gelatinized starch. The connection between starch and protein was tight, with the protein enveloping the starch. With one exception, no free water was visible; it was taken up by the gelatinized starch. Shortening was still visible although less distinctly after heat treatment in baking (Fig. 31). Gas vacuoles were not visible, probably because the crumb grain structure was destroyed by mounting in the specimen holder.

Findings discussed in this section are summarized in Table I.

DISCUSSION

Previous investigations of dough and bread structure by SEM and TEM were supplemented and complemented by the freeze-fracture technique. Each technique provides a different type of information: surface structure in SEM, diffraction of transmitted electrons through thin sections in TEM, and minimally treated structures at high magnifications in freeze fracture.

Our freeze-fracture method differs radically from the specimen-preparation method used for SEM by Khoo et al (1975), which involves freeze-drying rather than freeze-fracture.

Although freeze-fracture produces images more "lifelike" than other electron microscopic techniques, its use has several drawbacks. Interpretation can be difficult because only fractured surface features are observed. The images obtained are basically a series of shadows that must be related to the original structure. Comparisons of the freeze-fracture micrographs to light micrographs and thin-sectioned material (Bechtel et al 1978) facilitated our freeze-fracture interpretations, and many of our observations are based on them.

Appearances of structures of the main isolated components were compared with their appearances in dough and bread. Starch was the most consistent (least modified) ingredient; changes in shapes of the small and large starch granules (postulated by Khoo et al 1975) could not be detected even in fermented dough containing malt. Only in bread crumb were the starch granules modified (gelatinized) substantially; they lost their spherical shape, but not their granular ultrastructure.

The freeze-fractured isolated gluten had a sheetlike appearance. The TEM structure of whole gluten proteins described by Crozet et al (1974) was a smooth compact matrix that had fibrillar and granular zones associated with numerous lipid inclusions. In this study, the sheetlike structures of isolated gluten and of doughs and bread differed. But the small lipid inclusions in gluten were similar to those in the doughs and bread containing sugar.

Similarly, the structure of the isolated water-soluble fraction had no equivalent in the dough; however, in flour-water doughs (containing yeast and soy flour) the "dry clay soil" appearance in the water droplets and large water areas partially resembled isolated water-solubles.

Although TEM studies (Bechtel et al 1978, Khoo et al 1975, Simmonds 1972) showed a protein network composed of fibrils that interact with starch granules, SEM studies (Aranyi and Hawrylewicz 1969, Chabot et al 1979, Evans et al 1977, Varriano-Marston 1977) demonstrated a veil-like protein sheet enveloping

TABLE I
Summary of Freeze-Fracture Observations[a]

Dough Composition and/or Processing Stage	Starch Granule Water Coat	Water Distribution in Protein Network	Large-Water Area	Protein Distribution	Starch-Protein Interaction
Mixed dough					
FW or FWY	Broad	Droplets	"Dry clay soil"	Strands in coarse network	"Pearl chains"
FWSo	Broad	Droplets	"Dry clay soil"	Strands in network are stretched	"Pearl chains"
FWYS	Broad	Droplets	Contains "pearl chains"	Strands in network are stretched	"Pearl chains"
FWYSSh	Broad	Droplets	Contains "pearl chains"	Strands in network are stretched	"Pearl chains"
FWYSShSu	Narrow	Diffuse	Smooth	Transition between network and sheet	Little, with adhering strands
FWYSShSuM	Narrow	Diffuse	Smooth	Transition between network and sheet	Little, with adhering strands
Complete or complete + soy	Narrow	Almost no free water	Smooth	Sheet	Not detected
Fermented dough					
FWYS	Broad	Droplets	Contains "pearl chains"	Network	Fairly intensive, adhering protein strands
Complete	Broad	Droplets	Smooth	Network	Adhering protein strands
Bread crumb	...	...	...	Sheet, dense particle distribution	Tight connection

[a] F = flour, W = water, Y = yeast, S = salt, M = malt, So = soy, Sh = shortening, and Su = sugar.

the starch granules. In our study we observed a pattern of protein development as additional ingredients were added to the basic flour-water dough. The protein network, including water droplets, was transformed to a sheetlike structure after all ingredients were added. After sugar was added, a transition stage appeared between the two structures. At the same time, lipid inclusions became increasingly obvious in the sheetlike protein. Whether the sugar was directly causing these changes or if they were the result of sugar's cryoprotectant role, could not be determined from our data. Although the protein structures in the mixed and fermented flour-water-yeast-salt doughs did not differ, the sheetlike structure in the mixed complete dough was converted during fermentation into a network. This agrees with SEM results of Khoo et al (1975), who found that the veil-like protein in a mixed dough stretches and rolls up into fibrils during dough fermentation and proofing. In the bread crumb, dense protein sheets showed protein denaturation and water uptake by starch during gelatinization.

We observed starch-protein interaction in several samples. In the doughs containing salt and shortening, the connection was formed by thin "pearl chains." The extent of interaction decreased after sugar was added and, at the same time, thin protein strands adherred to the starch granules. After fermentation, however, the interaction intensified in the flour-water-yeast-salt dough. In the bread crumb, a tight connection formed between protein and starch.

Water distribution in dough and bread could be visualized by the freeze-fracture technique but not by TEM and SEM. The starch granules and yeast cells were coated by water, which is comparable to a definite separating space between starch granules and protein (Evans et al 1977). After sugar was added, the broad water layer around the starch granules narrowed and was accompanied by transformation of protein from a network to a sheetlike structure. The protein network enclosed water droplets, which became smaller and more diffuse after sugar was added and almost disappeared after oxidant was added (complete dough). Large water areas were visible in all doughs; in flour-water doughs, they contained soluble material and showed a weblike structure. After salt was added, the areas became smoother and the web seemed to be occupied by "pearl chains." After sugar was added, the large water areas were smooth. No change was detected in the large water areas after fermentation. In the bread, the water was taken up by the gelatinized starch and only the water surrounding the yeast cells was still visible. No vacuoles, reported by Bechtel et al (1978) on the basis of TEM, could be detected by freeze-fracture.

Several salient, novel, and (in part) unexpected findings resulted from this investigation. 1) The space between the starch granules was filled by gluten protein and large water areas. 2) Added ingredients caused protein development in mixed doughs from a network to a sheetlike structure; after sugar was added, a transition stage was visible. No change was detectable in protein development after fermentation in the flour-water-yeast-salt dough, but a dramatic change occurred in the complete dough, from sheetlike protein to a network. 3) Water distribution changed with protein development. The protein network included water droplets; the sheetlike protein contained irregularly distributed water. At the same time, the ultrastructure of the large water areas changed (from "dry clay soil," to "pearl chains," to a smooth surface). 4) Two types of protein-starch interactions were observed ("pearl chains" and thin strands), depending on the composition of the dough. The interaction was intensified after fermentation. 5) Although the study of isolated flour components (such as gluten) is warranted from a basic analytical viewpoint, flour components may undergo such profound modifications in a complex dough system that identifying them is impossible.

In summary, the freeze-fracture method proved to be a useful technique to investigate the structure of dough and bread. It should find application in determining the structure of other cereal products, including cookies and cakes.

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