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Hydrated Protein Fibrils from Wheat Endosperm

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ABSTRACT

Microscopic fibrils of hydrated protein are observed when thin sections or particles of wheat endosperm tissue are wetted with a drop of water. These protein fibrils are pulled from broken endosperm cells by the surrounding flowing solution and exhibit both viscous flow and elastic deformation. Interaction between starch granules and protein is evidenced by the adherence of starch granules to the surface of the protein fibrils which is clearly seen in scanning electron micrographs. The electron micrographs also indicate that the protein fibrils may be as small as 100 Å in diameter. The observed properties of the fibrils suggests that they are the structural elements which form the cohesive matrix of dough when worked mechanically.

Models for dough structure have relied upon gross macroscopic properties of doughs combined with the submicroscopic properties of protein molecules to provide a basis for their development. Only a few investigations (1-6) have studied the microscopic structure of dough in an attempt to bridge this enormous range in the size of the units upon which measurements were made, and all of these investigations relied upon dehydrated, fixed specimens. Consequently, observations could be made only of sequential changes in structure that could be fixed by the techniques used at the times selected to fix samples. We have overcome these limitations by studying the wetting of unfixed sections and particles of wheat endosperm while continuously recording the changes that occur on videotape.

By microscopically observing the hydration of a thin section of endosperm tissue as it is covered by a drop of water, we have seen fibrils of hydrated protein streaming out from the mass of endosperm, explosively at first, but with gradually diminishing velocity. The web of hydrated protein that spreads through the solution is peppered with large and small starch granules adhering to the fibril surface. The known fibrillar nature of α-gliadin (7) and its rheological properties following mechanical work (8) suggest to us that these endosperm fibrils are the structural elements which, when worked mechanically, form the macrostructure known as dough.

MATERIALS AND METHODS

Wheat kernels and flour used in all micrographs in this study were of the hard red winter variety Scout. However, no difference was found between this variety

and several others that were examined, including hard red spring, hard red winter, soft wheats, and durum wheats. The rye, Triticale, corn, rice, and barley also examined were all viable seeds.

Sections of endosperm were hand cut approximately 50 μ thick for hydration. Conventional light microscopic techniques were used to observe the hydration of the sections. A single drop of water placed directly upon the section resting on a microscope slide was sufficient to completely hydrate the section and provide excess water for the dispersion of the endosperm protein. Data were recorded on videotape through a standard television camera and finally transferred to 16-mm. movie film through a videotape to film transfer[1].

Specimens for scanning electron microscopic examination were freeze-dried samples of hydrated protein coated with carbon and gold to prevent charging of the specimen in the electron beam. The carbon and gold layer was approximately 200 Å thick. Our estimates of the size of the fibrils took into account this carbon-gold layer. Samples were frozen at -60°C., warmed to 0°C., and held at that temperature until dry. Some samples were frozen at -170°C. and held at -65°C. until dry. No differences were found for these two methods of sample preparation and no artifacts due to ice crystal formation were observed in either method.

RESULTS AND DISCUSSION

Endosperm sections from wheat kernels react rapidly with water, transferring the entire contents of endosperm cells to the aqueous phase wherever endosperm cell walls are broken. Cutting the endosperm cell wall at the end of the cell and exposing the cellular contents to water is sufficient to initiate the dispersion of the cell contents. Small fibrils of protein stream rapidly into the surrounding solution. The initial fibrils quickly develop into a linked network of fibrils as more protein is pulled from the cell and rapidly expand the volume they occupy. The rate of expansion slows gradually, but frequently continues until the entire contents of the cell are dispersed into the solution. The three-dimensional web of protein thus produced is highly interconnected, and a break in one fibril is reflected throughout the network. Starch granules, large and small, adhere to the surface of the fibrils. Fibrils which develop from the protein in the interior of the cell are frequently highly linked with those adjacent, resulting in a high density of fibrils. This linked web apparently results from the dispersion of the protein in a fibrillar form from the matrix protein deposits in the endosperm (Figs. 1 and 2).

Regardless of the location of the endosperm cell within the kernel, the reaction and apparent extent of fibril formation remain the same. We do not understand the driving force of the reaction, but it probably results from a combination of forces. Surface phenomena may be involved in the first interaction of the flour particle with water. However, since most of the endosperm cell contents are dispersed under the surface of the droplet, other forces must be considered. It is apparent that fibril elongation through flow and elastic stretching results from the viscous drag of the solution flowing away from the endosperm particle. This solution flow is rapid as indicated by rates of flow of free starch granules (100 μ per

[1]This film was shown at the 57th Annual Meeting, Miami Beach, November 2, 1972.

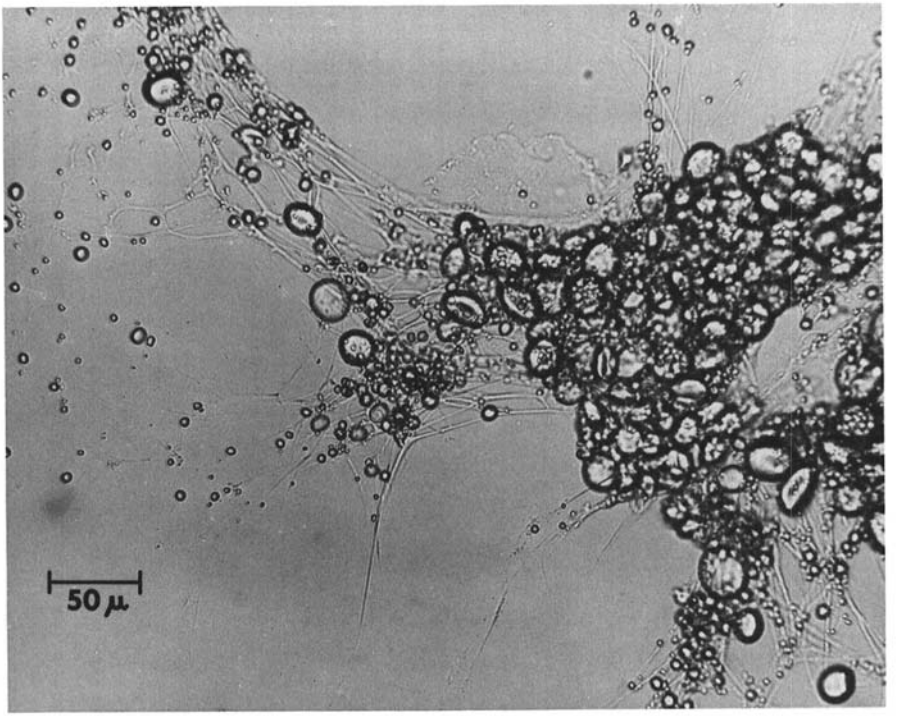

Fig. 1. A single flour particle wetted with a drop of water. Protein fibrils with adherent starch granules have increased the volume occupied by the particle nearly 20 times.

sec.) and must be convection currents generated within the water drop. The fibrillar properties of the hydrated protein are the phenomena to be considered in this paper, not the rapid solution flow away from the endosperm particle.

Identical results to those obtained with thin kernel sections are obtained with flour particles; fibrils form in all directions from the particles. The flow, and consequently the drawing out of the protein fibrils, can be restricted by high flour concentrations or any physical obstruction, such as covering the flour particles with a cover glass; the protein fibrils in the hydrated flour particles are readily seen by mechanically spreading the tissue with a microprobe. This demonstrates that the protein fibrils are not artifacts of the aqueous reaction in dilute solution. Once formed, the fibrils are very slow to dissolve and will persist in suspension for several hours.

The fibrils become smaller in diameter as elongation proceeds. If the solution flow is sufficiently rapid and viscous flow in the fibril is insufficient to relax the applied stress, the fibril breaks and exhibits an elastic component in the deformation. The elastic recovery may account for as much as 20% of the fibril elongation. The elastic component in the deformation decays but may not reach zero, as evidenced by fibrils that break after all apparent flow has stopped, yet still show elastic recoil of the broken ends. Furthermore, there is no retraction of the fibrillar web once it has formed, even though all flow has stopped.

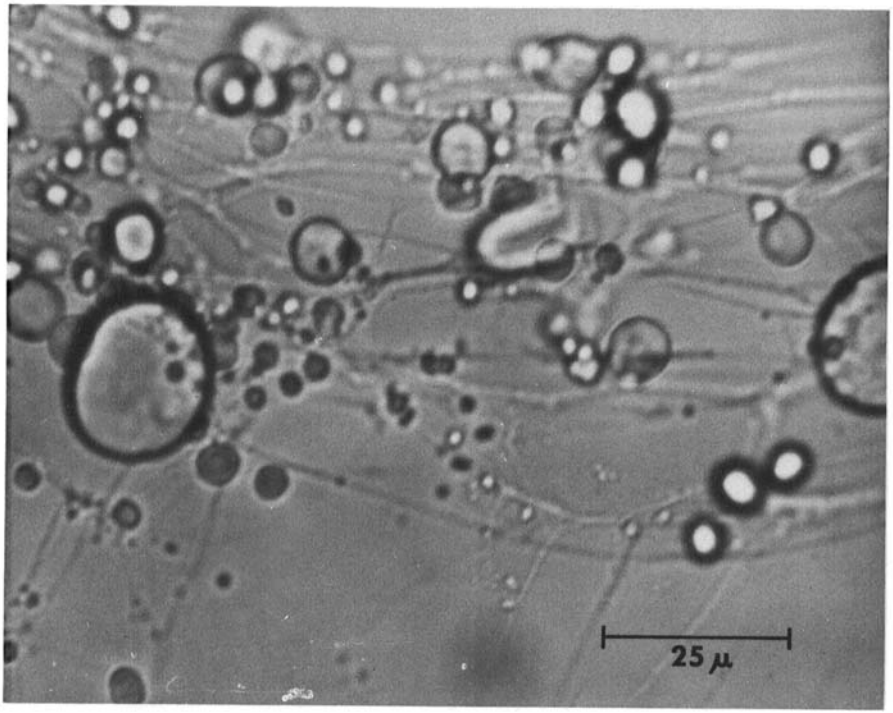

Fig. 2. A higher magnification of a portion of Fig. 1. The largest fibrils are approximately 1 μ in diameter. Starch granules vary from 1 to 20 μ in diameter.

All of the wheat endosperm protein seen in the light microscope exhibits these properties. Triticale and rye form fewer fibrils than wheat and are also the only other cereal grains which have any capacity to form a dough similar to wheat dough. No tendency for fibril formation was observed in corn, rice, or barley endosperm sections. Similarly, isolated wheat starch shows no tendency toward fibril formation, even though some protein may remain on the surface of the starch granules.

There is an interaction between the starch fraction and the protein fibrils as evidenced by the adherence of starch granules to the surface of the fibrils. The forces exerted on the starch granules by the flowing solution may contribute to the drawing out of the protein fibrils. In cases where a fibril is observed to break and recoil elastically, a starch granule may be dislodged from the fibril and float free in solution even though other granules on the same fibril remain adherent. This variation in the strength of interaction between the starch granules and protein fibrils may only result from a variation in surface contact area. While starch acts as a filler in the dough system, the interaction of the starch granule with the protein fibrils indicates that the starch granule surface characteristics should affect the uniform dispersion of the starch in the protein fibril matrix. Any change in the starch granule surface characteristics which would weaken the adherence of the

granules to the protein fibrils would probably decrease the uniformity of the final texture that is desired in baked goods.

Flour hydration occurs very rapidly. Wetting a single particle of flour in a droplet of water requires less than 0.05 sec. for complete hydration as measured by the initiation of fibril formation. Within 5 sec. the hydrated protein web spreading from the particle reaches its maximum volume with a 20-fold or greater increase over that of the initial particle. Adjacent flour particles spreading similar protein networks interact to form a continuous interacting system that can be worked mechanically to form a doughlike mass.

Scanning Electron Microscopic Observations

When the protein fibrils are fixed by rapid freezing and then freeze-dried, the fine structure of the fibrils and their interactions with starch can be examined. The greater resolving power of the scanning electron microscope as compared to the light microscope demonstrates that there are finer and many more fibrils than can be seen in the light microscope. The surface characteristics of the fibrils are also visible. Rather than continuous strands of drawn out protein, the fibrils appear to be drawn out from sheets of protein (Fig. 3) and have torn fragments of the sheets along their length. Some of these fragments may provide interaction sites for the

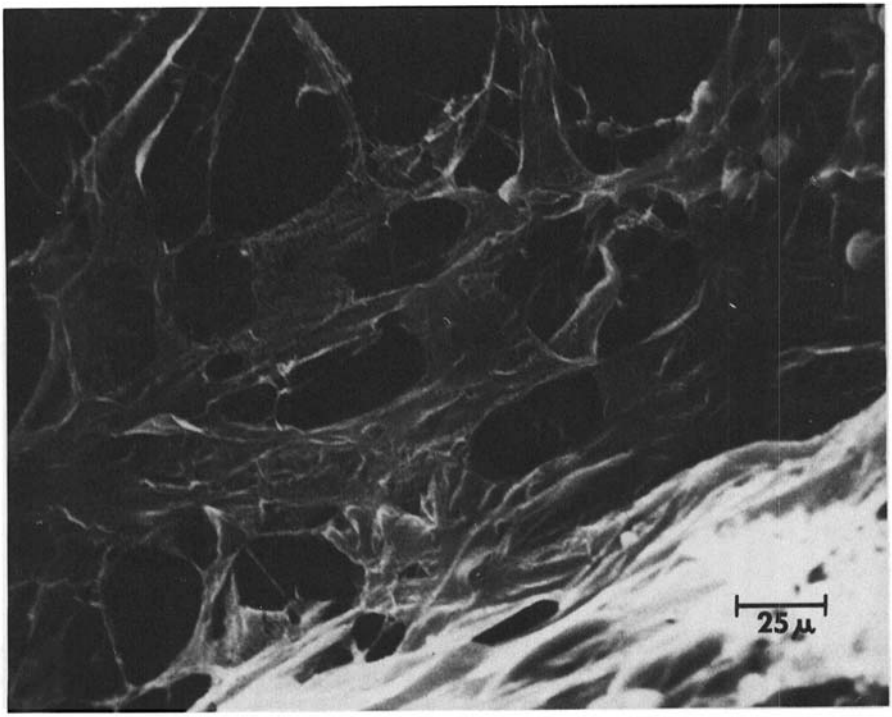

Fig. 3. A scanning electron micrograph of a freeze-dried endosperm particle after wetting. The larger mass of the original particle is in the foreground with sheets of protein in varying stages of breakdown extending from it. Small starch granules are visible through the protein sheet.

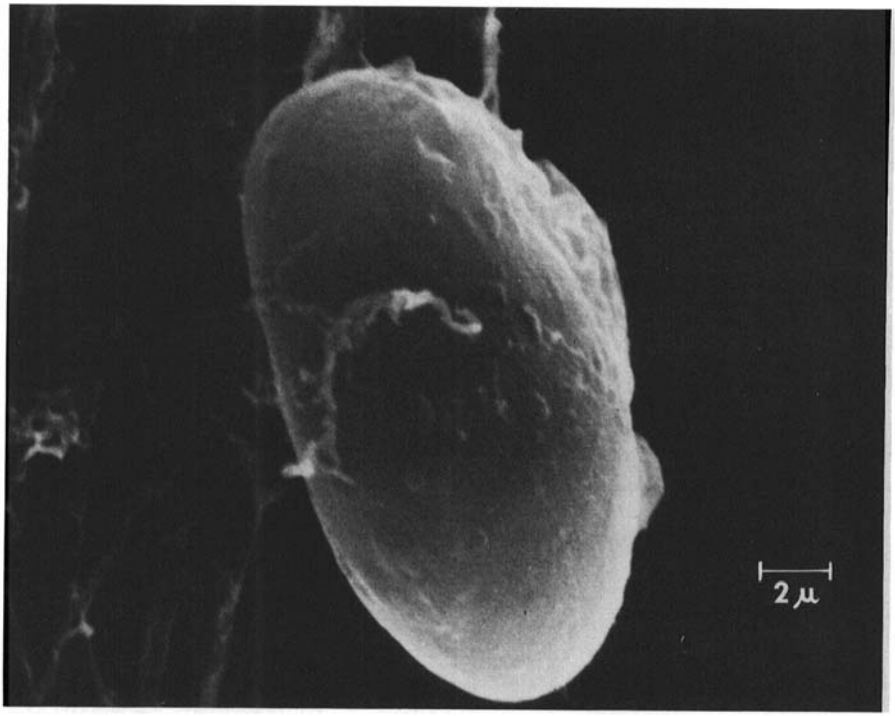

Fig. 4. A small starch granule associated with some smaller fibrils. A portion of one fibril is seen wrapped around the surface of the granule.

starch granules (Fig. 4). Fibril interaction with the surface of the starch granules may involve only a small portion of the starch surface (Fig. 4) or it may nearly envelop the granule (Fig. 5).

In several micrographs (not shown), fibrils with diameters in the range of 100 to 300 Å could be observed. These fibrils are small enough to be composed of α-gliadin fibrils (7). Large fibrils could be composed of lateral aggregates of these smaller fibrils, which may also provide cross-links between the larger aggregates. Resolution in the scanning electron micrographs is not sufficient to permit an evaluation of this hypothesis.

GENERAL DISCUSSION

Our previous experience with the rheological properties of solutions of α-gliadin fibrils (8) suggested that the protein fibrils produced upon wetting endosperm tissue might contribute to dough properties. Solutions of α-gliadin (greater than 3% protein) in its fibrillar form have been shown to change from a highly fluid solution to a gel with the application of mechanical work. Characteristics which parallel dough development, stability, work hardening, relaxation, and dough breakdown have been demonstrated for the α-gliadin gel (7). Furthermore, the interacting α-gliadin fibrils in the gel, or developed form, exhibit complete elastic recovery of

applied stress prior to relaxation of the stress through viscous flow. At any point on the stress relaxation curve after flow begins to dissipate the stress, the residual stress can be recovered as elastic energy.

It has not been possible to isolate single fibrils and analyze them to determine which proteins form the fibrils. While similar fibrillar forms have not been demonstrated for the other gliadin proteins, all gliadins are similar in both chemical and physical properties. It therefore seems reasonable to postulate a fibrillar form for gliadin proteins. Fibrils have been described for the endosperm protein surrounding starch granules (9), but attempts to demonstrate a fibrillar form for the bulk of the endosperm protein have failed. Grosskreutz, in describing a fibrillar aspect of freeze-dried gluten sheets (3), suggested that lipid was essential for the plastic properties of the protein. While no attempt was made in these studies to demonstrate the presence or absence of lipid, other workers (10) have shown that free lipid is a contaminant of flour from the aleurone and bran during milling. Also, the disruption of compact lipoprotein membranes permitting the interaction of the lipid with storage protein as suggested by Simmonds (6) seems unlikely under the conditions of this study. The interaction between storage protein and lipid to form high-molecular-weight aggregates as suggested by Simmonds and Wrigley (11) is not pertinent to this study, since their work involves complexes formed during gluten isolation. Furthermore, preliminary experiments with defatted flour indicated lipid

Fig. 5. A protein sheet interacting with a large starch granule and enveloping nearly one-third the starch granule surface. Smaller starch granules are visible throughout the protein matrix.

does not prevent the observation of fibrils when the flour is wetted. We therefore consider the elasticity and plasticity of the protein to be a property of the protein and independent of lipid.

Fibrillar structures derived from endosperm tissue have been reported by Seckinger and Wolf (10), but their technique did not demonstrate the viscous or elastic properties of the fibrils or the interaction of protein and starch.

The rapid convective flow of the solution does provide some mechanical working of the protein. However, since this work results from the laminar solution flow that draws out the fibrillar structures, it is unlikely that mixing of the various endosperm components occurs to any significant extent.

This study demonstrates that prior to dough development the endosperm protein possesses elastic properties. While the properties of the endosperm protein may be significantly altered by the addition of lipid and other dough additives, the fundamental properties which allow dough formation reside in the protein.

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