A Note on the Flocculation Mechanism of the Zeleny Sedimentation Test

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The Zeleny test for breadmaking quality of wheat flour (1) involves the measurement of the volume of precipitate formed upon suspension of flour in a dilute lactic acid:isopropanol solution (final concentrations: 1.3% lactic acid, 6.7% isopropanol); the greater the volume of precipitate, the better the flour quality. In 1969, Muller and co-workers showed that the Zeleny test depended on the flocculation or "line settling" of flour particles rather than on gluten swelling as previously had been supposed (2,3,4,5). In spite of extensive microscopic and microelectrophoretic studies, these workers could not decide whether flocculation was caused by long-range particle interaction or by molecular bridging.

In 1973, Bernardin and Kasarda (6) described the behavior of flour particles when wet with excess water in a microscope slide. With many particles, hydrated protein fibrils streamed out explosively at first but rather more slowly later.

It occurred to us that these fibrils might take part in the flocculation in the Zeleny test. Experiments in our respective laboratories show this to be true. Particles in the floc are bridged by protein fibrils, so that flocculation is clearly associated with a bridging process. Hydrated flour particles, with fibrils extending into the surrounding solution, adhere to one another when they collide evidencing strong attractive forces between the fibrils. Muller and co-workers had not seen these fibrils because they have nearly the same refractive index as the Zeleny reagent in which they are suspended.

Figure 1 shows a flour particle in Zeleny solution A (water + bromophenol blue). For this photograph, several flour particles were placed in a flow cell 0.5 mm. thick and irrigated with the solution. The cell was then placed under the microscope and the photograph taken. The bromophenol blue effectively stains the protein, which is almost transparent in water alone. Figure 2 shows a flour particle treated as above, but then flushed with Zeleny solution A + B in the appropriate ratio (Zeleny solution B is the lactic acid-isopropanol reagent). This treatment converts the bromophenol blue to the pale yellow form and renders the fibrils almost colorless. For Fig. 2, the blue color was restored by again irrigating the particle with Zeleny solution A. Apart from the color change, there was no noticeable change in the physical appearance of the fibrils upon reverting to solution A.

Figure 3 shows material that was subjected to the standard Zeleny test. A small drop of the suspension of the voluminous floc was placed on a microscope slide. It was then diluted with additional solution (A + B) and covered with a coverslip. Excess solution was removed with filter paper and a drop of dye placed at the edge of the coverslip. The diffusing dye gradually stained the protein. Aniline blue black (C.I. 20470) in acetic acid:methanol was used for the photographs taken at the USDA laboratory. The same effect was obtained in the Leeds laboratory with Coomassie brilliant blue R250 (C.I. 42660). No noticeable structural change occurs as a result of the staining.

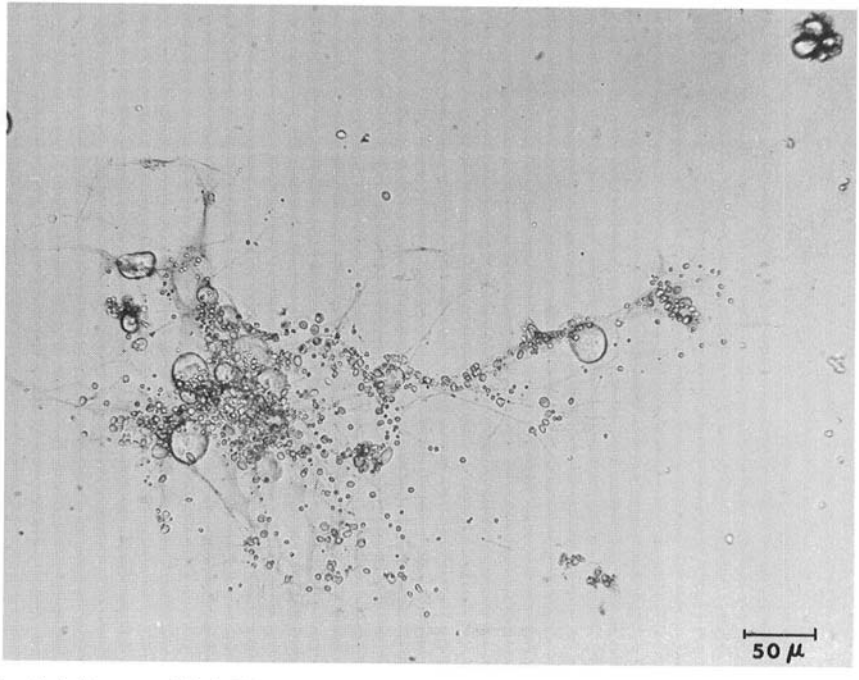

Fig. 1. A flour particle in Zeleny solution A (water + bromophenol blue). Sheets and fibrils of protein are clearly visible spreading into the surrounding solution.

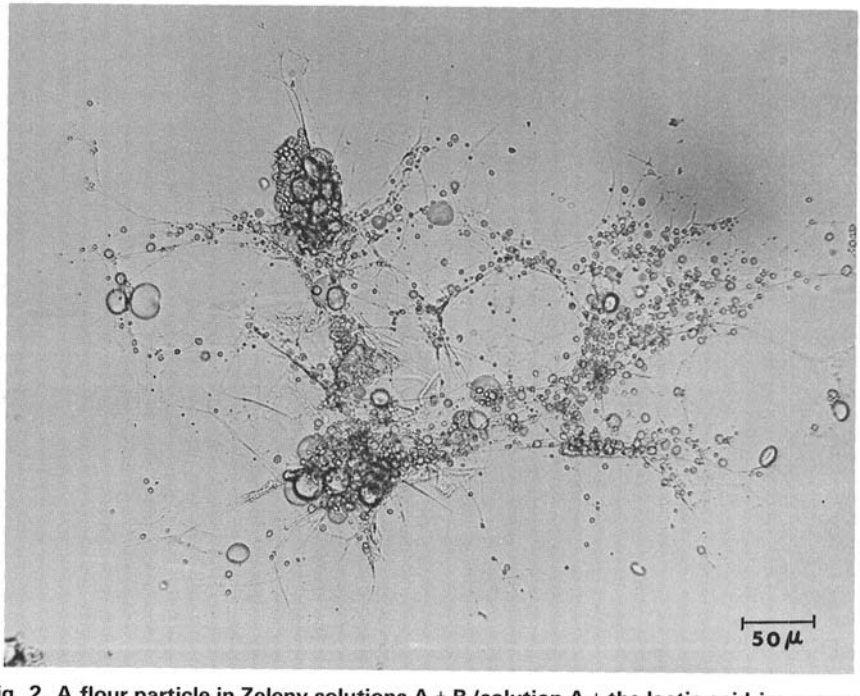

Fig. 2. A flour particle in Zeleny solutions A + B (solution A + the lactic acid-isopropanol reagent). Protein fibrils are evident and are stable in the reagent for hours.

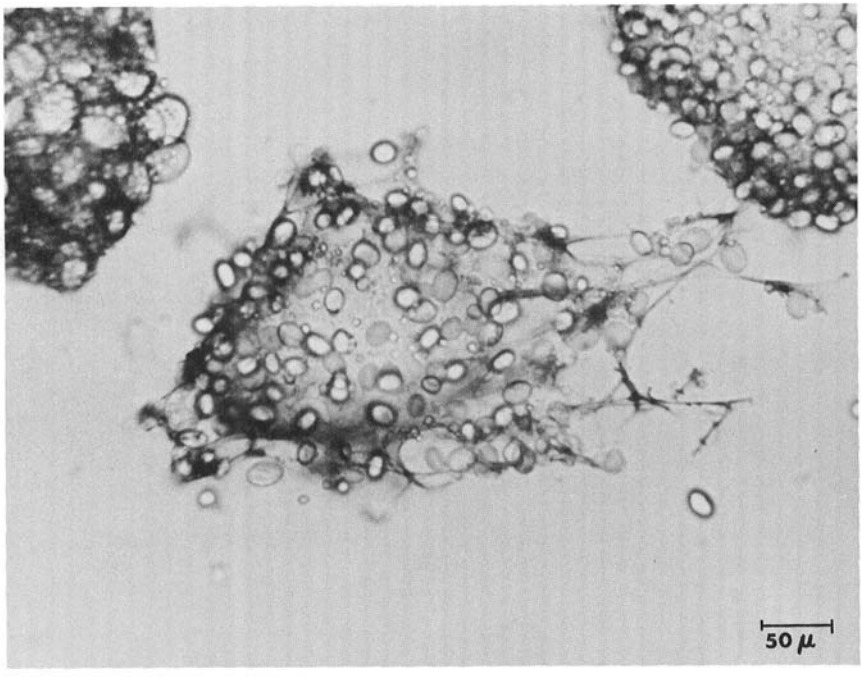

Fig. 3. A portion of the voluminous floc taken from a Zeleny sedimentation test showing the interacting fibrils bridging between flour particles.

It appears that the Zeleny sedimentation test depends mainly on the flocculation of the large flour particles (3). These particles are modified by the reaction of the flour with, first, water and, subsequently, the lactic acid-isopropanol reagent; they then interact by bridging through the protein fibrils. The solubilization of flour protein by water and lactic acid solutions is well known. Some proteins are not solubilized by the reagents in the Zeleny test. These proteins, in sheet and fibril form, stabilize the voluminous floc measured in the test. It is likely that these are the same proteinaceous components which are seen in developed doughs as fibrillar strands of interacting protein (7) and account for the validity of the Zeleny test.

The quantitative aspects of the mechanism are not clear and are receiving further attention.

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[Received March 18, 1974. Accepted June 13, 1974]