MICROSCOPIC EVALUATION OF BREAD FORTIFIED WITH CONCENTRATED PLANT PROTEINS

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ABSTRACT

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Bread was baked from wheat flour with up to 2% of the flour replaced by vital gluten and 17% of the flour replaced by concentrated plant proteins, including soy flour, sunflower concentrate, fababean concentrate, and field pea concentrate. Previous studies showed that the supplemented breads showed decreased loaf volumes, compact or coarse crumb grains, and firm textures that were resistant to compression. Samples of these breads were observed by light microscopy and scanning electron microscopy. The supplemental proteins were shown to disrupt the well-defined protein-starch complex observed in the wheat flour bread. In addition, small pores were observed in the thick cell walls of the supplemented breads, and these pores may allow gases to escape from the structure during baking.

Examination of the microstructure of wheat flour doughs and bread has been helpful in determining the changes that occur in physical structure during the various stages of bread preparation. Mixing has been shown to result in an even distribution of ingredients and the development of gluten into a continuous, fibrillar network (1,2). By stretching out the dough through sheeting rolls, large masses of protein were converted into long, thin fibrils aligned parallel to the direction of flow through the sheeting rolls. In addition, starch granules became oriented parallel to the film surface during proofing and gelatinization. It has been postulated that the strength of dough films was due to the strong adherence of protein to starch and suggested that a continuous protein film appeared to separate the starch granules in the bread crumb (2).

The scanning electron microscope (SEM) permits observation of three-dimensional structures, and the specimens need not be cut into thin sections prior to examination. SEM evaluation of wheat flour doughs showed that the protein matrix formed a smooth enveloping, veil-like network stretched over the starch granules (3–5). The starch granules were evenly distributed throughout the entire surface. Other researchers observed wheat flour dough in the stressed condition and noted that the gluten seemed to form thin sheets over the starch granules on some surfaces and fiber-like structures on others (6). They suggested that the fiber formation was caused by the rupturing and rolling back of a sheeted structure. Others showed a unidirectional orientation of the swollen strands in bread and proposed that the veil-like protein and the underlying starch formed a cohesive mass (4).

Concentrated plant proteins can be incorporated into bread to increase both the quality and quantity of protein at a low cost. In this manner, a diet can be significantly improved without changing eating habits. Extensive research has been conducted on the influence of soy protein on bread quality (7,8), but other concentrated plant proteins have also been used, including sunflower (9), fababean (10), and field pea (11). In all cases, these foreign proteins caused

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deterioration in bread quality characteristics such as loaf volume, crumb texture, and crumb compressibility (12). These characteristics were not due to lack of fermentable components, since each dough formula had the ability to produce large quantities of carbon dioxide during fermentation. Therefore, the bread cell structure appeared unable to retain this gas during baking. A recent study indicated that incorporation of single-cell protein results in disruption of the elastic gluten structure as observed by SEM (5).

The object of our study was to compare the microstructure of wheat flour bread (70% extraction rate) with the microstructure of high-protein bread supplemented with soy flour, sunflower concentrate, fababean concentrate, or field pea concentrate. Both light microscopy and SEM techniques were used to observe changes in the physical structure of bread due to the incorporation of concentrated plant protein into the formula.

MATERIALS AND METHODS

Light Microscopy

Modifications were made on the light microscopy methods previously described (13,14). Cubes approximately 1 cm^3 in size were cut from the top, center, and bottom of the center slice of loaves 18 hr after baking. The bread pieces were immediately placed in labeled glass bottles and covered with cool (5° C) 4% aqueous glutaraldehyde buffered to pH 6.8 ($Na_2HPO_4 + NaH_2PO_4$) for a minimum of 72 hr.

The fixed bread samples were taken from the fixative and cut with a sharp scalpel into cubes approximately 0.5 cm^3. These cubes were frozen in position on a microtome specimen holder with the aid of Cryoform embedding media and fitted to a retracting microtome. The frozen bread cubes were sectioned at a thickness of 8–10 μm and the sections were carefully transferred to albuminized slides and air-dried.

The proteins were stained with 0.1% aqueous Ponceau 2R (C.I. No. 16150) containing 3–4 drops of 1*N* H_2SO_4/50 ml stain (15). The specimen was stained for 10 min, then thoroughly washed with distilled water, leaving the proteins colored red. Yeast cells were stained with methylene blue (C.I. No. 52105) according to the method of Fink and Kuhles (16). The stain solution contained 200 mg/L of methylene blue, 27.2 g/L of KH_2PO_4, and 0.071 g/L of Na_2HPO_4, and remained in contact with the specimen for 5 min. The specimen was then thoroughly rinsed.

The samples were observed with a Leitz ortholux photomicroscopic system. The images were recorded on 35 mm Kodak high contrast copy film and printed on Kodak F-4 photographic paper.

Scanning Electron Microscopy

Small (*ca.* 0.25–0.50 cm^3) pieces of freeze-dried bread were broken or cut from the center portion of the loaves and mounted on small disk-shaped metal sample holders with a silver adhesive. All samples were coated with a thin layer (300–400Å) of a gold-palladium alloy evaporated onto their surfaces in a Hummer vacuum evaporator.

The samples were observed with a Cambridge Stereoscan Mk II SEM operated at 20 kv. Images were recorded on Kodak Plus-X Pan film (ASA 125)

using a Linhof Super Rolex camera. The photographs were printed on Kodak F-4 paper.

Bread Samples

Five samples of bread were evaluated. These breads were prepared according to the methods previously described (12) and contained wheat flour, concentrated plant protein, and vital gluten in ratios of 100:0:0 for wheat flour bread; 83:15:2 for soy, fababean, and field pea bread; and 86:12:2 for sunflower bread.

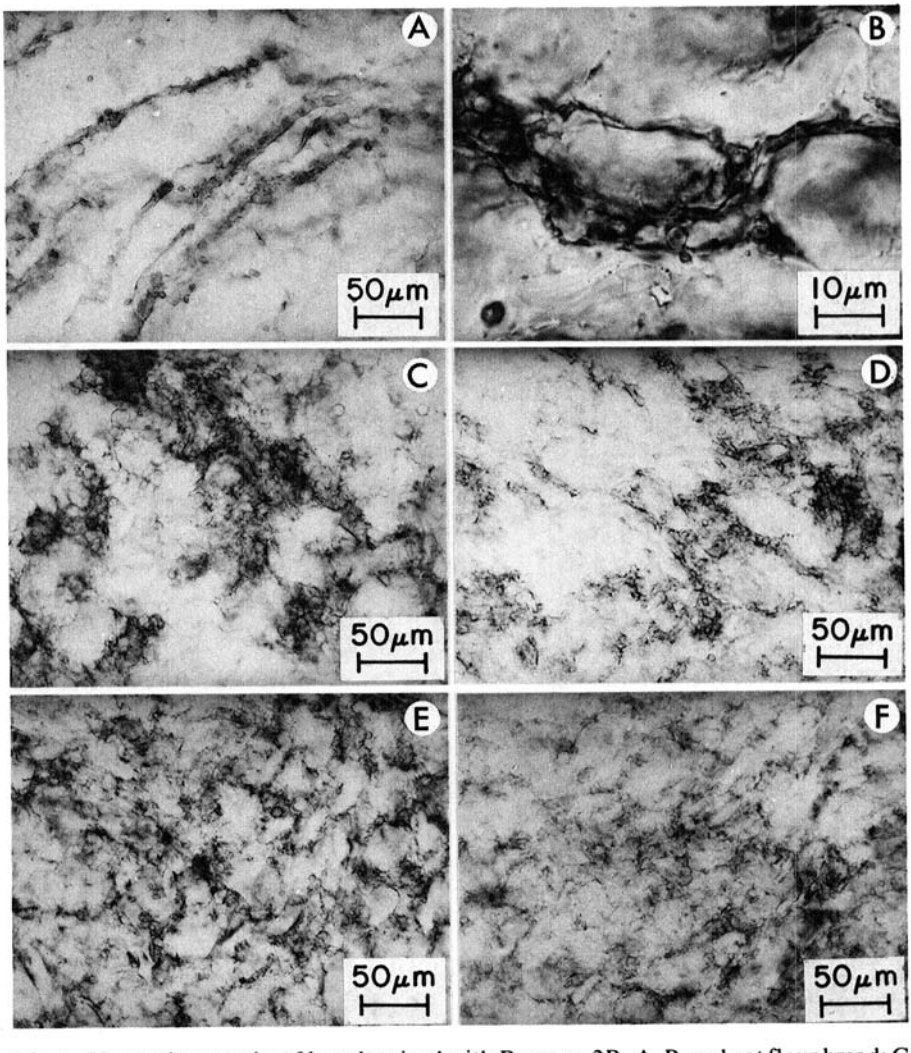

Fig. 1. Photomicrographs of bread stained with Ponceau 2R. A, B = wheat flour bread; C = soy bread; D = sunflower bread; E = fababean bread; F = field pea bread.

RESULTS AND DISCUSSION

Light Microscopy

The protein in wheat flour bread was distributed in linear arrangements that were observed as fibrillar formations (Fig. 1A). The majority of protein fibers were distributed unidirectionally and were cross-linked. The width of the fibers was fairly consistent, but showed areas of high protein density at interconnecting junctions. Due to the nature of the sampling technique, confirming that the fibrillar formations were cross sections of proteinaceous sheets was not possible. Intact starch granules, identified by iodine staining, are visible in the high-magnification photographs and appear as opaque, somewhat circular configurations (Fig. 1B). The starch granules were oriented in close proximity to the protein fibers and took on irregular shapes to fit the spaces available. A similar orientation of components was previously shown in wheat flour dough and bread (1,2).

Some fibrillar formation occurred in soy bread, but the structure shown in Fig. 1C was most typical of the protein distribution. While the protein tended to be organized into linear areas, these areas were internally disorganized. Similarly, the protein in sunflower bread showed limited tendency toward organized fiber formation (Fig. 1D). Although the protein was, in general, unidirectionally oriented, no regularity was observed in some areas of protein accumulation. The protein distribution in fababean and field pea bread showed no fiber formation and no organized arrangement (Fig. 1E, F). A continuous protein network was observed, and no areas were shown to be predominantly starch or protein. High magnification of sunflower and soy bread (photos not shown) indicated that the starch granules were uniformly dispersed in the proteinaceous zones and that their shape had been altered to fill the space in a manner similar to that previously shown for wheat flour bread (Fig. 1B).

Ponceau 2R is a general protein stain and does not differentiate the wheat gluten protein network from the proteins present in the concentrated plant proteins. Since the protein distribution in soy and sunflower bread showed some similarity to wheat flour bread, however, one can postulate that the proteins from the two sources do become associated. This association may be physically (entrapment) or chemically (electrostatic bonds) induced.

The yeast in wheat flour bread was shown to be distributed throughout the structure, but concentrated in the proteinaceous zones (Fig. 2A), which may be caused by electrostatic attraction. Generally, the yeast cells were spherical or ellipsoidal in shape (Fig. 2B). The spaces between the protein fibers were filled with starch granules and can be individually identified (Fig. 2B). Yeast cells in soy and fababean bread were distributed throughout the structure, but were associated with the protein (Fig. 2C, 2E). Individual yeast cells and starch granules in close proximity to the protein can be identified (Fig. 2D, 2F). Photomicrographs showing yeast-protein distribution in sunflower and field pea bread (not shown) were similar to those for soy and fababean bread, respectively.

Scanning Electron Microscopy

Observation of wheat flour bread and bread supplemented with concentrated plant proteins generally showed two distinct areas that are referred to as thin-cover and thick-cover areas. This difference appears to be due to the extent to

which protein is deposited over the starch granules.

In the thin-cover areas of wheat flour bread, the clearly visible starch granules were embedded under a continuous sheet believed to be the protein that constituted the gluten proteins in the dough state (Fig. 3A). A similar network was noted in wheat flour dough (3). Whereas the starch granules that those workers showed had diameters ranging from 5 to 10 μm, the granules in Fig. 3A were 10–15 μm. Swelling due to baking probably accounts for the larger starch granules in the baked bread. The intermediate areas shown in Fig. 3A were

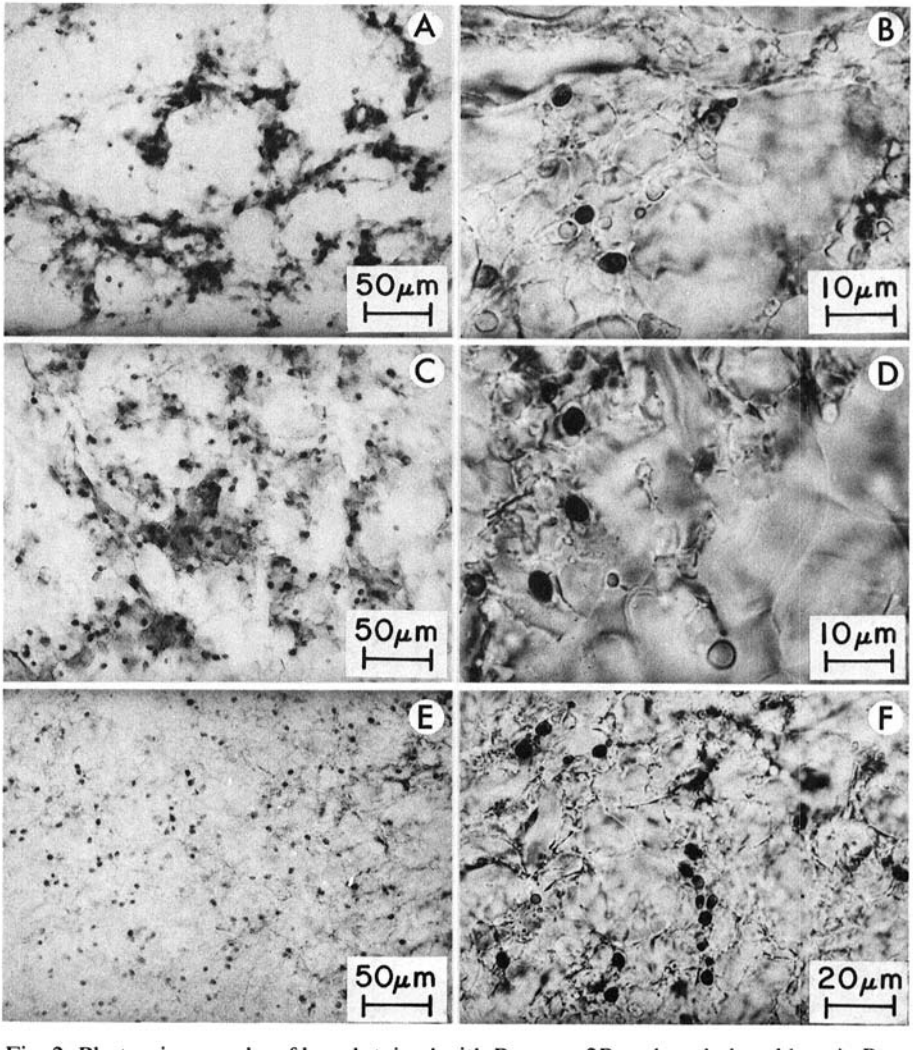

Fig. 2. Photomicrographs of bread stained with Ponceau 2R and methylene blue. A, B = wheat flour bread; C, D = soy bread; E, F = fababean bread.

not smooth and extended as would be expected for a proteinaceous sheet under stress, but were shown to be rather uneven and had a relaxed appearance. A similar phenomena was noted when wheat flour doughs were compared in their stressed and relaxed states (6).

In contrast to the wheat flour bread, the soy, sunflower, fababean, and field pea breads showed porous, broken surfaces in the thin-cover areas (Fig. 3B-E). The surface sheets appeared to have been subjected to a stress greater than they

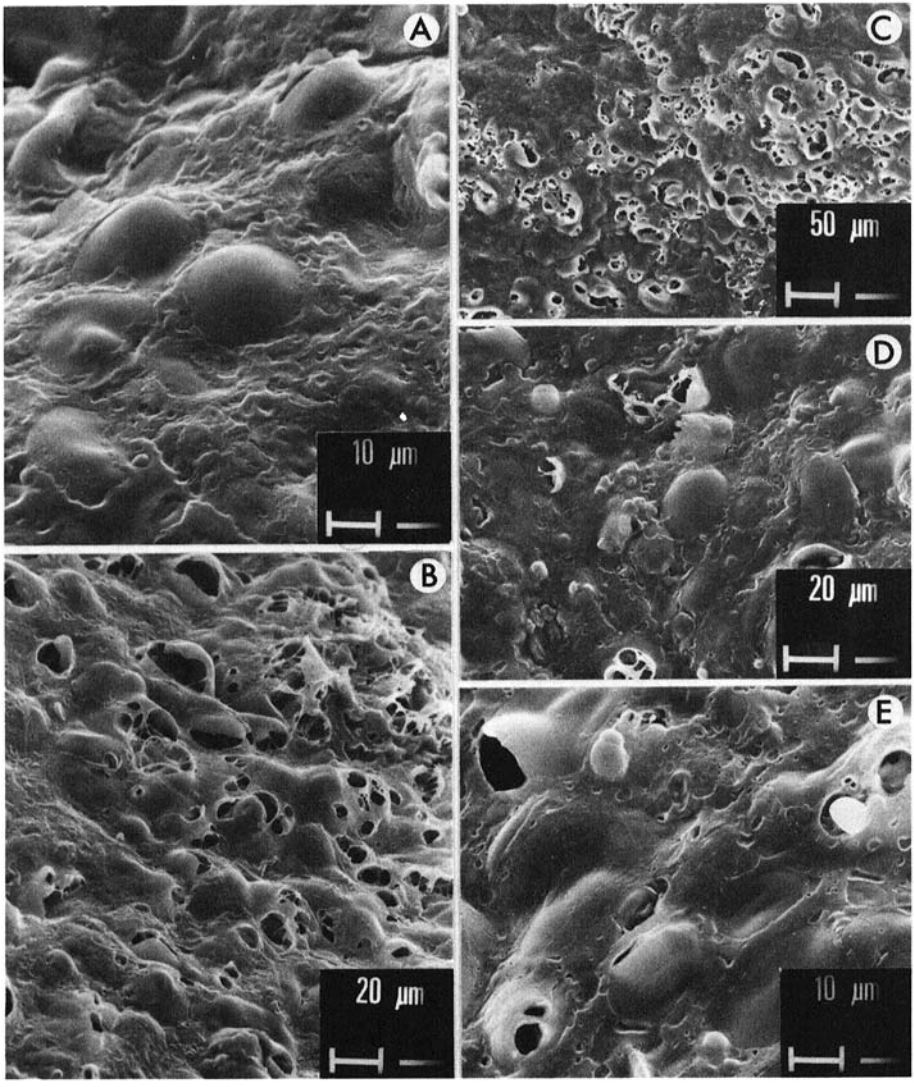

Fig. 3. Scanning electron micrographs of bread observed in thin-cover areas. A = wheat flour bread; B = soy bread; C = sunflower bread; D = fababean bread; E = field pea bread.

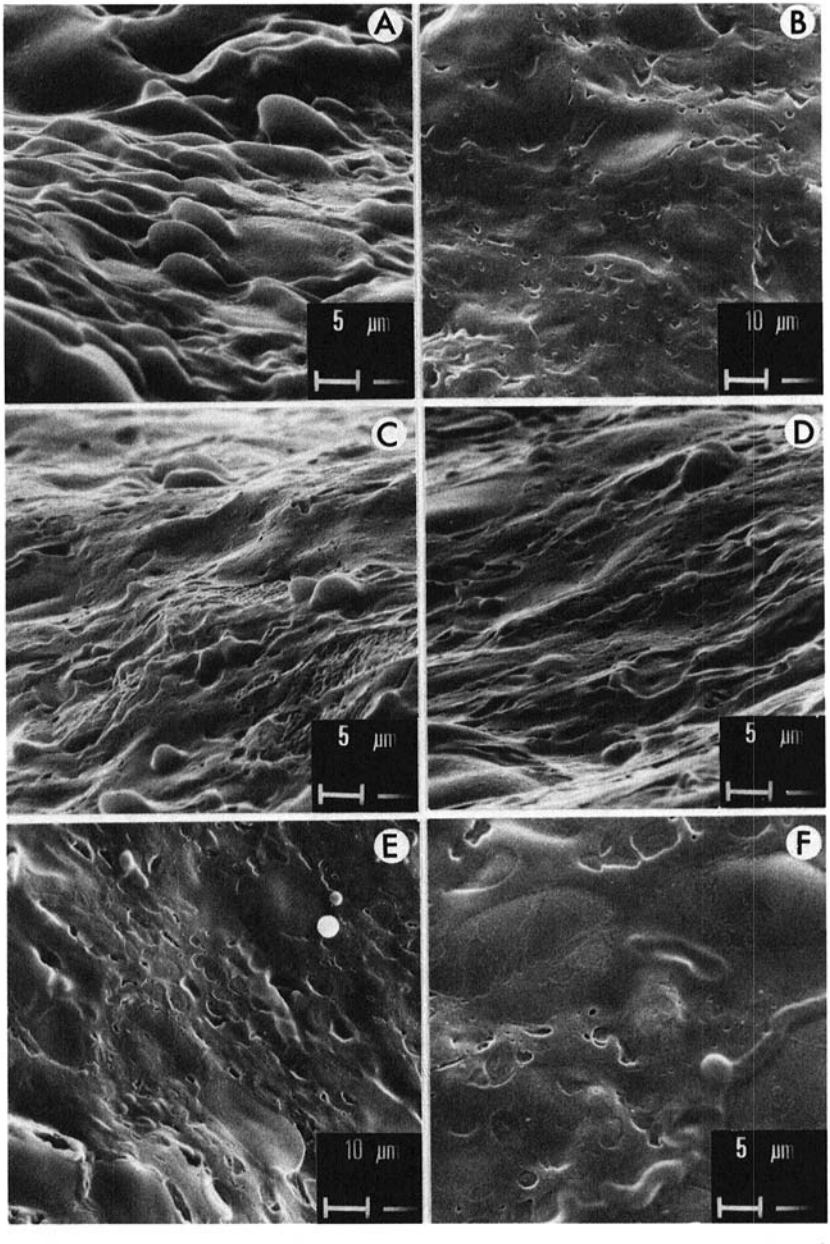

Fig. 4. Scanning electron micrographs of bread observed in thick-cover areas. A = wheat flour bread; B = soy bread; C = sunflower bread; D = fababean bread; E, F = field pea bread.

could withstand. This resulted in rupture at the weakest points, which appeared to be the sections covering starch granules.

In areas referred to as thick-cover areas, the wheat flour bread appeared to have a smooth, even surface that flowed over various contours, presumably starch granules (Fig. 4A). The soy, sunflower, fababean, and field pea breads showed a dense surface with little evidence of embedded starch granules (Fig. 4B-E). Small pores ranging in size from 0.25 to 1.0 μm in diameter were observed in each supplemented bread and were typical of those shown in Fig. 4F. These pores

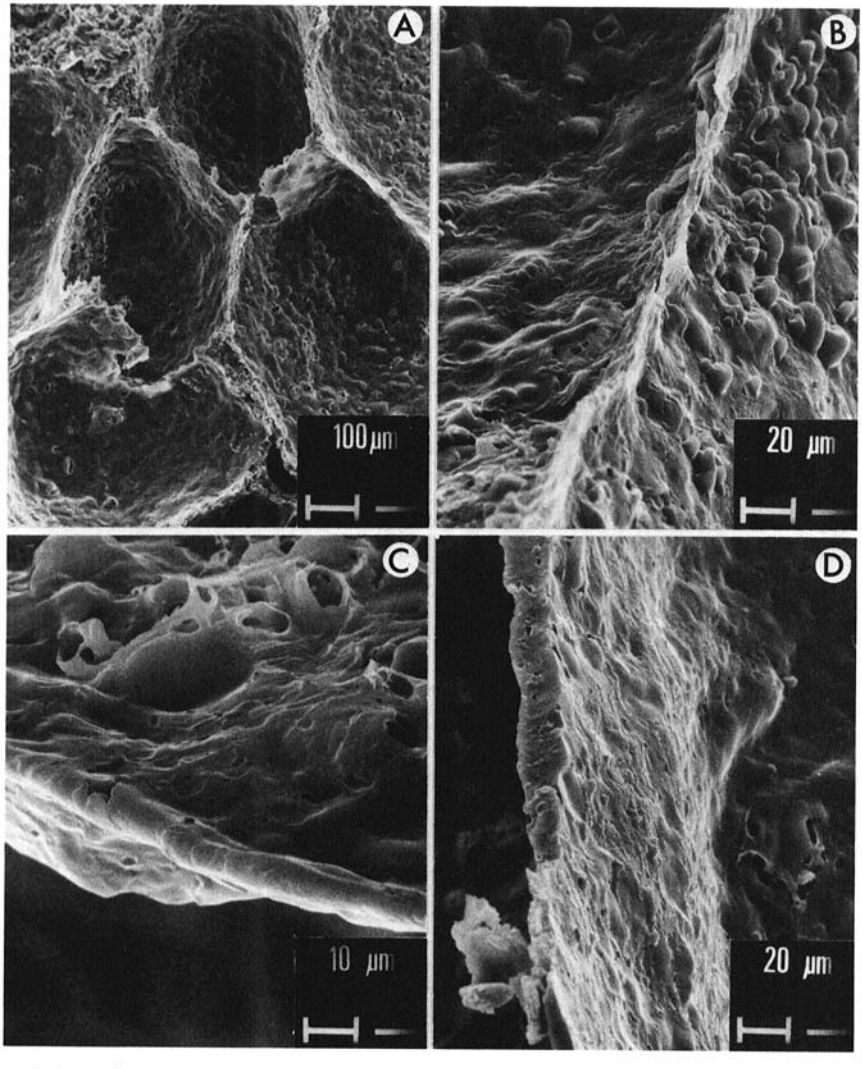

Fig. 5. Scanning electron micrographs of bread cell walls. A, D = sunflower bread; B = wheat flour bread; C = soy bread.

were not observed in wheat flour bread and therefore may indicate that a weak gluten structure was present in the dough and was not capable of withstanding the expansion and pressure that occur during baking. These pores would premit the gases that cause oven-rise to escape and result in smaller loaves. The high gassing power reported for these supplemented doughs (12) indicated that the formulas could support sufficient yeast fermentation. The resulting low loaf volumes could be explained by the poor physical structure noted in these photographs.

The walls that divide the bread cells were observed to form a pattern typical of the structure shown for sunflower bread (Fig. 5A). When observed at higher magnification, the walls of wheat flour bread averaged 2–5 μm in thickness and were composed of a series of sheets that appeared to be laminated into a composite structure (Fig. 5B). The complex walls of the supplemented breads were 5–20 μm thick and generally appeared to have no pattern of composition (Fig. 5C, D). The thick cell walls explain in part the dense loaf and low crumb compressibility that was observed in these breads (12).

CONCLUSIONS

Concentrated plant proteins were shown to disrupt the well-defined protein-starch complex observed in wheat flour bread. The light microscopy results indicated that the protein in wheat flour bread was organized into linear arrangements separated by starch granules, whereas the proteins in the supplemented breads showed no regular or linear associations. The influences of fababean and field pea were more pronounced than were soy or sunflower. In all cases, the yeast was associated with the protein, but the wheat flour and concentrated plant proteins could not be differentiated by staining techniques.

The SEM micrographs indicated that the concentrated plant proteins caused small pores and a ruptured cell structure in bread. In addition, the cell walls of supplemented breads were thick, complex structures compared with the thin, sheeted walls in the wheat flour bread. A weak gluten structure may account for these characteristics and would result in depressed loaf volumes, irregular crumb grains, and a firm crumb resistant to compression.

The changes in internal bread structure noted in this study may account for some of the deteriorations in breadmaking quality caused by incorporating concentrated plant proteins into bread.

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