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LIGHT AND SCANNING ELECTRON MICROSCOPY OF WHEAT- AND RYE-BREAD CRUMB. INTERPRETATION OF SPECIMENS PREPARED BY VARIOUS METHODS

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Abstract

The crumb of bread baked from wheat flour, rye flour, and rye meal was examined by light- (LM) and scanning electron-microscopy (SEM). Whereas in the wheat bread the crumb is held together by a matrix of denatured protein, in the rye bread crumb highly expanded starch granules fulfill that role. Fracturing freeze-dried crumb provided different information than sectioning prior to freeze-drying. In the first case, little damage was caused to components of outer surfaces of vacuoles. In the second case, the protein matrix and starch granules were broken. At the same time, the presence of micropores in the material surrounding the vacuole was observed and confirmed the findings from LM of sections of the bread crumb. Examination by SEM of residues of bread crumb macerated to wash out soluble starch demonstrated the presence of a residual coherent structure of apparently denatured gluten proteins in wheat bread. In rye bread there were only few similar, less coherent, structures.

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**KEY WORDS:** Wheat bread, Rye bread, Bread crumb, Scanning electron microscopy of bread crumb, Light microscopy of bread crumb

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Mention of firm names or trade products does not constitute endorsement by the USDA over others not mentioned.

Introduction

We have recently reported (Pomeranz et al., 1984a, b) SEM studies on the structure of doughs and breads from rye, wheat-rye, and rye flours and meals. Those studies confirmed that the structure of wheat flour doughs is governed primarily by the contribution of the gluten components. Interaction between the starch and the gluten proteins strengthens somewhat the dough structure. In the wheat-rye dough, additional contributions are made by the rye gums. The structure of the acidic dough from rye flour or meal is governed by contributions of gums, aggregates of starch granules, starchy endosperm particles, and bran particles.

The majority of the walls of vacuoles of the crumb in bread baked from wheat flour are structured as a well developed, fine network of protein strands and membranes which surround and interact with starch granules. The swollen and expanded starch granules support the main gluten structure. In addition to the well developed crumb structures, there are present areas of brittle walls in which no protein-starch interaction can be observed. The crumb of rye bread is characterized by a smaller number of closed vacuoles and heavy walls, which are composed primarily of starch granules. Those starch granules are highly modified-expanded and embedded in a gummy matrix.

In the course of those investigations it became clear that some of the interpretation depended on the manner in which the specimen was prepared. Similar concern was expressed by Chabot (1979), Chabot et al. (1979), and Varriano-Marston (1977).

This communication, therefore, examines the interpretation of our previous findings in light of the above concerns and provides new information, by using additional methods, to observe the structure of bread crumb.

Materials and Methods

Bread

Formulations and procedures used in preparation of the wheat and rye flours and meal bread were described elsewhere (Pomeranz et al., 1984a, b).

### Light Microscopy

Bread crumb was sectioned with a freezing microtome table, (Leitz Co., Inc.) with cooling aggregate for object table and knife cooling. Pieces, about 5 mm long, were cut from freshly baked bread and frozen without fixation, in an embedding medium for frozen tissue specimens (O.C.T. compound) at  $-20^{\circ}\text{C}$  on the object table of the microtome and sections 10  $\mu\text{m}$  thick were prepared. The sections were glued to glass slides painted with a thin layer of glycerol-gelatin, stained for protein with Xylidin Ponceau (Pomeranz and Shellenberger, 1961) or for starch with iodine, and observed under a Zeiss light microscope.

### Scanning Electron Microscopy

Two methods of sample preparation were used:

A. Chunks of crumb were removed from freshly baked bread cooled to room temperature, frozen at  $-20^{\circ}\text{C}$  in a Leibold Heraeus GT 3 freezer, and freeze-dried. Small pieces of the freeze-dried crumb were mounted on specimen holders with a special glue (Leit C) in such a manner that the original surfaces of the freeze-dried crumb removed from the freshly baked bread could be examined. The mounted pieces were sputter-coated with gold. The preparations were viewed and photographed in a Leitz AMR 1,600 T scanning electron microscope at an accelerating voltage of 20 kV.

B. Slices of freshly baked bread were frozen at  $-20^{\circ}\text{C}$ , freeze-dried, and broken. The newly broken surfaces were examined by SEM as in Method A.

### Results and Discussion

Examination under the light microscope makes it possible, through staining, to determine semiquantitatively the distribution of protein and starch. Figures 1 and 2 indicate that distribution of protein in the crumb of both the wheat and rye bread is not uniform. There were considerably more areas that stained for protein in the wheat bread crumb than in the rye bread crumb. In addition, the wheat bread crumb had several areas of high protein concentration distributed at random throughout the crumb. The general impression is that whereas in the wheat bread crumb the protein matrix holds the crumb together, in the rye bread crumb no such coherent matrix is present. The rye starch granules are more modified and expanded than the wheat starch granules, probably because of the lower gelatinization temperature of rye starch under baking conditions. The proximity of highly expanded starch, even in the micrograph of the rye bread crumb, does not prevent the recognition of well delineated, individual starch granules. To the extent that protein is present, it surrounds the starch granules in the rye bread crumb and forms a matrix in which rye starch granules are embedded. A similar conclusion was reached during observation under the LM (not shown) of iodine-stained preparations. Protein comprised the major matrix in the bread crumb of wheat bread and starch in the crumb of rye bread. The results were, however, not as clear-cut due to

the non-specific staining by iodine of non-starchy components, especially in the rye bread. In the case of bread made from whole grain rye meal, an additional contributor to the crumb is the presence of chunks of starchy endosperm and bran (not shown).

Scanning electron micrographs of the wheat and rye bread crumb are shown in Figs. 3-10. The greater depth of field observed in scanning electron micrographs presents a good overview of the structure of the bread crumb in wheat (Fig. 3) and rye (Fig. 7) bread. In samples prepared by Method A (surfaces cut before freeze-drying), one can see in areas surrounding the vacuoles the arrangement and distribution as well as modification-expansion of individual starch granules and compare them with, apparently, less modified starch granules (Figs. 3, 5, 7, and 9). In agreement with the micrographs from LM (Figs. 1 and 2), the starch is substantially more modified-expanded in rye than in wheat bread crumb. In wheat (Fig. 5 - high magnification), the starch granules are clearly embedded in a protein matrix. On the other hand, it is more difficult to discern the outline of the individual starch granules in the inner structure of the vacuole in rye bread (Fig. 9 - high magnification). This is due to the more extensive expansion-modification of the rye starch granules, but also to the fact that they may be covered by a layer of gums, proteins, and soluble starch.

Samples prepared by Method B (surfaces cut after freeze-drying) differ substantially from samples prepared by Method A. Whereas the outer surfaces of the vacuoles remain relatively intact, considerable damage was caused to the starch, proteins, and other components inside the vacuole walls (Figs. 4, 6, 8, and 10). A comparison of the micrographs of crumb prepared by Method A (Figs. 3, 5, 7, and 9) with the micrographs of crumb prepared by Method B (Figs. 4, 6, 8, and 10), indicates that the former show little and the latter show a considerable amount of micropores. Those micropores are the result of minute vacuoles in the walls surrounding the larger vacuoles. They can be seen only after the walls of the vacuoles are fractured. They can be seen also in the preparations examined under LM. The possibility that some of the micropores are the result of shrinkage during freeze-drying, cannot be excluded. Thus, the information obtained by the two methods of sample preparation complements the other. Useful information can be obtained by examination of micrographs at high magnification. Method B shows the folding and layer-like stratification of starch in the walls of the vacuoles as well as a partial cover of a protein matrix. The results of micrographs for Method B, as expected, parallel those for LM of their sections from frozen material. This information is particularly useful in examining the vacuolar wall structure of rye bread. Whereas it is difficult to conclude from Fig. 9 about the manner in which the starch granules are held together, Fig. 10 demonstrates the "spot welding" interaction at fairly regular intervals, that is responsible for the structure of the crumb of rye bread.

The findings were also confirmed by scanning electron micrographs of bread crumb macerated with water to remove the soluble starch prior to mounting. In the case of wheat flour bread (Fig. 11), even after the soluble starch was washed out there remained a residual coherent matrix of the denatured gluten. In the case of rye bread, a less coherent structure was left as the main components were washed out (Fig. 12).

#### References

- Chabot, JF. (1979). Preparation of food science samples for SEM. Scanning Electron Microsc. 1979; 111: 279-286, 298.
- Chabot, JF, Hood, LF, Liboff, M. (1979). Effect of scanning electron microscopy preparation methods on the ultrastructure of white bread. Cereal Chem. 56, 462-464.
- Pomeranz, Y, Meyer, D, Seibel, W. (1984a). Wheat, wheat-rye, and rye dough and bread studied by scanning electron microscopy. Cereal Chem. 61, 53-59.
- Pomeranz, Y, Meyer, D, Seibel, W. (1984b). Rasterelektronenmikroskopische Untersuchungen über die Struktur von Weizen (Mehl) -, Weizenmisch-, und Roggenteigen und Broten. (Investigations on the structures of wheat-, wheat mixed- and rye and rye doughs and breads by scanning electron microscopy). Getreide, Mehl, Brot. 28, 138-146.
- Pomeranz, Y, Shellenberger, JA. (1961). Histochemical characterization of wheat and wheat products. II. Mapping of protein distribution in the wheat kernel. Cereal Chem. 38, 109-113.
- Varriano-Marston, E. (1977). A comparison of dough preparation procedures for scanning electron microscopy. Food Technol. 31(10), 32-36.

#### Discussion with Reviewers

R. Moss: "Freshly" baked bread--some indication of whether or not the bread was cooled would be useful in helping the reader assess likely artefacts associated with processing i.e. hot or warm bread is difficult to sample without causing artefacts on the cut surface. Also, more details of the freezing process--this is also not clear in the Cereal Chemistry paper i.e. air frozen, blast or still air?  
 Authors: Chunks of crumb were removed from freshly baked bread, cooled to room temperature. They were frozen in a Leibold Heraeus GT 3 freezer. The freezer is equipped with an air-suction device.

R. Moss: The term "spot welding" is not very informative. The comment re Fig. 9 seems equally applicable to Fig. 10 except that Fig. 10 is at higher magnification i.e. no information is given as to the nature of the components responsible for the 'spot' welding.

Authors: The magnifications in Figs. 9 and 10 are approximately the same.

R. Moss: No mention is made of the protein content of the flours used in these experiments. If they are the same as used for the 1984(a) Cereal Chemistry article, the appreciably lower protein content of the rye flour compared to the wheat flour may be responsible for the markedly different protein matrix in the LM of rye bread (Fig. 2) i.e. very little protein is apparent. In the reviewer's experience, the accumulations of protein seen in Fig. 1 are very dependent on protein content (as well as degree of development) and breads from low protein wheat flour have an extremely fine and delicate protein matrix.

Authors: The protein contents were 12.1% and 9.4% (both dry matter basis) in the wheat and rye flours, respectively. A decrease in protein content will decrease the extent of staining with Xylidin Ponceau. We do not believe, however, that the differences in protein distribution (Figs. 1 and 2) and in coherence of the protein matrix (Figs. 11 and 12) are due to differences in protein content, only.

R. Moss: The maceration experiments are interesting but why do the authors feel that the differences they observed are due to the washing out of soluble starch (as stated in the abstract), rather than a difference in the fragility of the crumb? The latter would be more related to the different nature of the protein matrix.

Authors: The differences were not due to maceration; they were made visible as a result of washing out of starch.

R. Moss: Are the artefacts associated with Method B due to shrinkage during freeze drying or due to shrinkage during freezing? Did the authors investigate other, more rapid freezing methods (e.g. isopentane cooled liquid N<sub>2</sub> or N<sub>2</sub> slush)? Freeze-fracturing the samples might also have provided helpful information.

Authors: We have not tried various freezing temperatures and have cited work of others (Chabot, 1979 and Varriano-Marston, 1977) in this respect. We do not believe that Methods A and B differed in their effects on shrinking.

Reviewer No. 2: The only difference I could see between Method A and Method B in the scanning electron microscopy was that in A original surfaces were viewed, and in B freeze-dried-fractured surfaces were seen. While I would predict some differences in these two surfaces, I would not expect the type of differences illustrated. Also, freeze-dried bread is very fragile, so keeping track of original versus fractured surfaces can be difficult. I thought that this was the reason for two separate protocols, because in fact both original crumb surface and fractured dried crumb surface can be revealed in one freeze drying step.

Authors: We presented only a small part of micrographs. Practically all wheat bread samples treated by Method B produced micrographs represented in Figs. 4 and 6; all rye bread samples

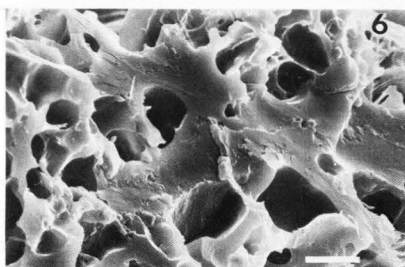
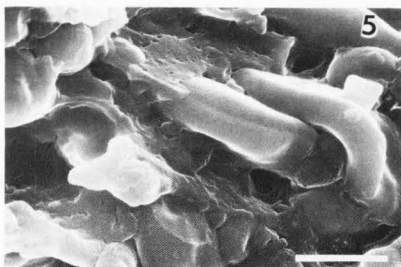
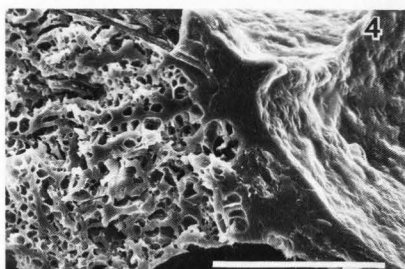
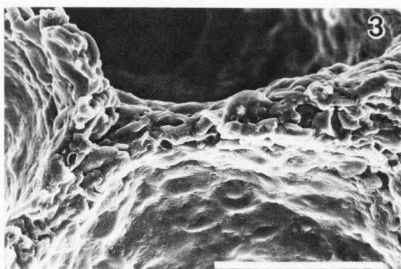
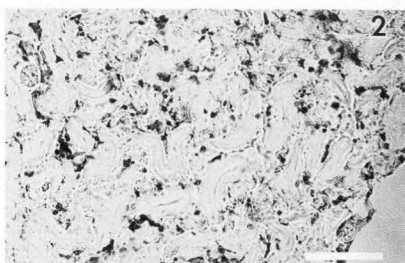
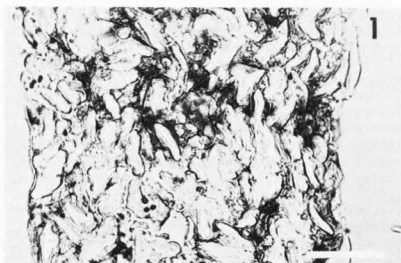


Figure 1. LM of a cross section through the wall of a vacuole of wheat bread crumb. Bar = 100  $\mu$ m.  
Figure 2. LM of a cross section through the wall of a vacuole of bread crumb from rye flour. Bar = 100  $\mu$ m.  
Figure 3. SEM of crumb of wheat bread, Procedure A. Bar = 100  $\mu$ m.  
Figure 4. SEM of crumb of wheat bread, Procedure B. Bar = 100  $\mu$ m.  
Figure 5. SEM of crumb of wheat bread, Procedure A. Bar = 10  $\mu$ m.  
Figure 6. SEM of crumb of wheat bread, Procedure B. Bar = 10  $\mu$ m.

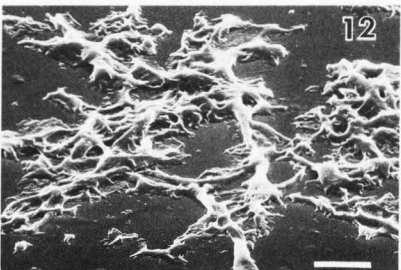
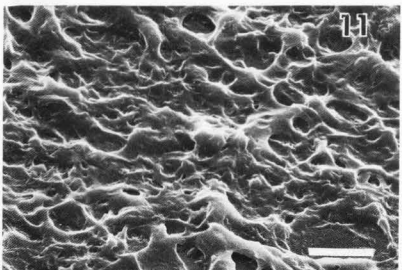
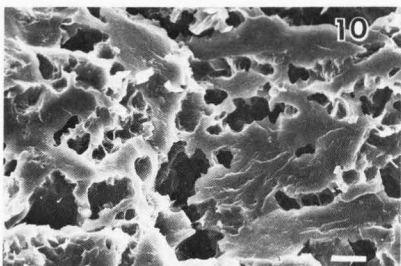
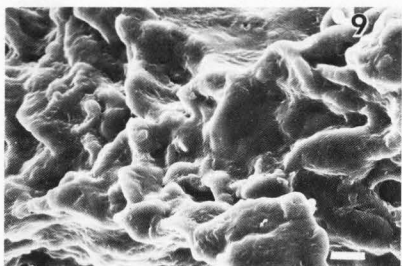
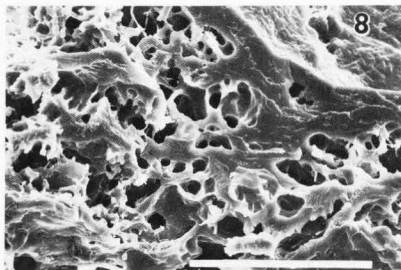
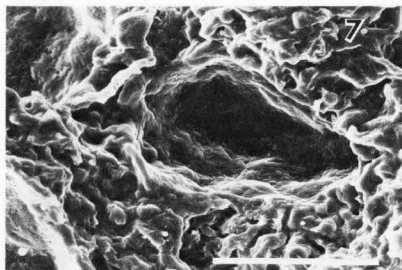


Figure 7. SEM of crumb of rye bread, Procedure A. Bar = 100  $\mu\text{m}$ .  
Figure 8. SEM of crumb of rye bread, Procedure B. Bar = 100  $\mu\text{m}$ .  
Figure 9. SEM of crumb of rye bread, Procedure A. Bar = 10  $\mu\text{m}$ .  
Figure 10. SEM of crumb of rye bread, Procedure B. Bar = 10  $\mu\text{m}$ .  
Figure 11. SEM of water-macerated crumb of wheat bread. Bar = 10  $\mu\text{m}$ .  
Figure 12. SEM of water-macerated crumb of rye bread. Bar = 10  $\mu\text{m}$ .

treated by Method B produced micrographs represented in Figs. 8 and 10.

Reviewer No. 2: Examination of the micrographs showed very great differences between the two protocols, but I did not understand why the B method produced such different images, with loss of detail of starch granules. It appeared that these images looked more like a freeze dried gel than like bread. Was it possible that in Method B the bread had become wet at some time? In rereading the light microscopy section, had the imbedding medium U.C.T. (which I am not familiar with) been used for scanning electron microscopy preparation as well?

Authors: It is our belief that the matrix and starch structure in samples prepared by Methods A and B differed significantly and consistently, irrespective of starch fracture. The samples were not imbedded for SEM. It is unlikely that the samples picked up large amounts of water during freezing.

Reviewer No. 3: Why do you think freezing at  $-20^{\circ}\text{C}$  will not damage the matrix and swollen starch granules during freezing?

Authors: Artefact formation as a result of ice crystal formation during freezing at  $-20^{\circ}\text{C}$  cannot be excluded. It is possible that use of lower freezing temperatures ( $-40$  or  $-60^{\circ}\text{C}$ ) should be investigated.

Reviewer No. 3: What were the relative volumes of the different breads? What was the relative amount of air cell structure in the breads? Could these differences account, in part, for the amount of matrix found/unit area of crumb observed? (This discussion is useful even when published earlier.)

Authors: The specific volumes of the rye bread were 1.9 to 2.4  $\text{g}/\text{cm}^3$  and of wheat bread 3.3 to 3.7  $\text{g}/\text{cm}^3$  (See Bruemmer, J. Getreide, Mehl, Brot. [1971], **25**, 125-128; [1972], **26**, 234-236).

Reviewer No. 3: Can the authors describe the differences in starch size, shape, swelling temperatures? Can one or two micrographs be included from previous work?

Authors: Das Getreide, Part I. Verlag Paul Parey, Berlin, (1966), pp. 28-30; small starch granules (up to 7.5  $\mu\text{m}$  in wheat and rye) comprise about 90% of the total number; wheat starch granules are up to 45  $\mu\text{m}$  and rye starch granules up to 60  $\mu\text{m}$  in diameter. The beginning average gelatinization temperatures are  $60^{\circ}\text{C}$  for wheat and  $56^{\circ}\text{C}$  for rye starch and the average final gelatinization temperatures are  $88^{\circ}\text{C}$  for wheat and  $62^{\circ}\text{C}$  for rye starch.