A Note on Scanning Electron Microscopy of Flours and Doughs[1]

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The microscopic structure of the wheat kernel and of flour has always been of great interest to cereal chemists. Light microscopy, transmission electron microscopy, and, to a lesser extent, X-ray diffraction have been used by many investigators (1–11) to pursue the subject. These experimental approaches, also applied in our laboratories, have been broadened by the use of a new technique, scanning electron microscopy.

The operating principle of the scanning electron microscope (Stereoscan; Cambridge Instrument Co., Ltd., England) is different from that of the transmission electron microscope. Is the latter, the sample is placed in the path of the electron beam, and the transmitted beam, which has been changed by absorption, contrast, and scattering effects of the sample, is examined. In the scanning electron microscope, the surface of the sample is scanned with an electron beam, and the image created by the secondary electrons emitted from the surface is observed. The image is produced on the screen of a cathode-ray tube connected to the scintillator-photomultiplier system that detects the secondary electrons. Photomicrographs of the images observed can be made instantly with a Polaroid camera (12).

The advantages of the scanning electron microscope include its very large depth of focus (generally as large as the field of view), the possibility of viewing much larger samples than in the conventional electron microscope, and easier sample preparation. No thin-sectioning or replicating methods are necessary. The surface of the sample can be examined directly without any special treatment, thus ensuring the absence of artifacts, a possible danger in more complex preparation methods. The sample is viewed at an angle, and therefore shadowing of the surface is inherent in the technique. Nonconductive materials, however, have to be coated with a uniform layer of evaporated metal to prevent the build-up of a negative charge on the surface by the primary electrons. The resolution of the scanning electron microscope is usually better than 500 A but can be as good as 200 A under appropriate conditions.

Figure 1 shows scanning electron micrographs of flour milled from hard winter wheat grown in Kansas and of dough samples mixed from this flour with distilled water in a 1:1 weight ratio. The special character of these micrographs is strikingly evident in the unusual depth of field, resulting in an almost three-dimensional effect.

For viewing, the samples were mounted on small disk-shaped metal sample holders with an aerosolized adhesive. Flour was dusted onto the surface, and the excess was removed with compressed air blown over the sample holder. For dough, the small sample sheets were pressed on the metal

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sample stubs that had previously been sprayed with adhesive and were dried in a vacuum desiccator after they were mounted. All samples were coated with a thin layer (about 300–400 A) of a gold-palladium alloy evaporated onto their surfaces in high vacuum.

For comparison, micrographs of flour and dough samples at similar magnifications are displayed side by side. In Fig. 1, A shows flour at relatively low magnification. Here individual flour particles can still be recognized as broken-up endosperm tissue. Lentil-shaped and round starch grains can be seen clearly. Surrounding the starch grains and distinctly different from the starch is a structural element with rough, broken, and curled edges; this is the proteinaceous matrix.

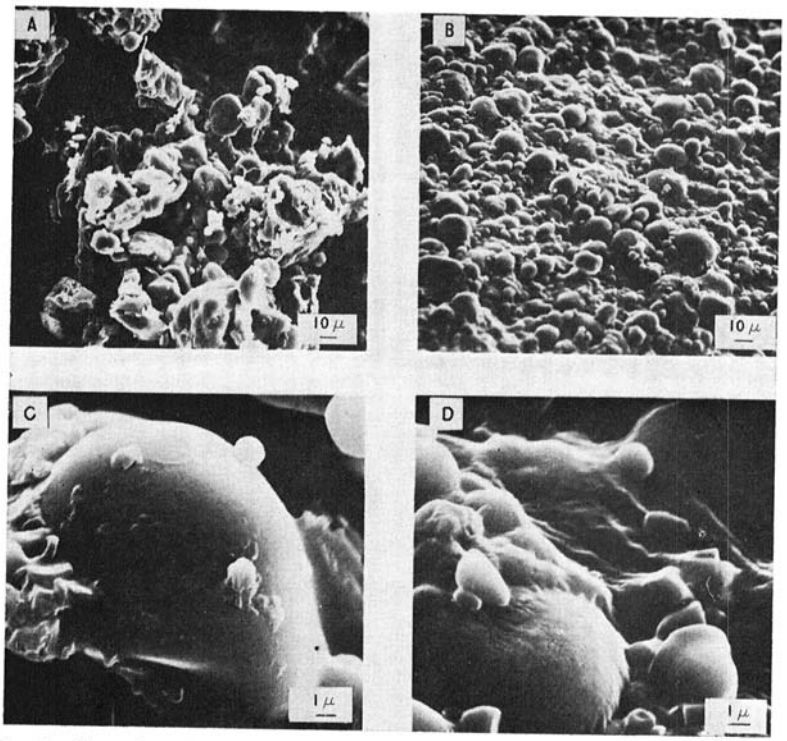

Fig. 1. Scanning electron micrographs of (A and C) flour and (B and D) dough samples at various magnifications.

In contrast, in the dough sample shown in B, starch grains are distributed more evenly over the entire area, and the edgy, rough protein matrix has been transformed into a veillike structure that seems to be stretched over and around the starch kernels. This is in agreement with the general concept of dough formation, according to which, during hydration and mechanical agitation, the protein and lipoprotein components of flour form a viscoelastic network that surrounds and envelops the starch granules.

When viewed at higher magnifications (C and D), the surface character

of both samples is seen in more detail. The starch grains of the flour sample shown in C reveal an interesting feature: pieces with different structural formation are attached to the smooth surface of the starch kernels. This structural element is believed to be the "adhesive protein" described by Hess (4). According to his theory, this protein component of flour is closely associated with the phospholipids, plays an important role in dough formation, and is structurally different from the "wedge protein" which occupies the interstitial spaces between starch grains.

Although some micrographs were taken at magnifications as high as 30,000×, they did not reveal any additional information. We hope, however, that in future work the resolution of the samples can be increased to such an extent that more can be learned from these experiments.

In conclusion, scanning electron microscopy is a very promising new tool for cereal research. It is believed that, in combination with light microscopy and transmission electron microscopy, this new tool will have valuable new applications in the field.

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