Lipid Distribution in the Protein Matrix of Wheat Endosperm as Observed by Electron Microscopy[1]

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ABSTRACT

Free and bound lipids were extracted from hard red spring wheat flour. Electron micrographs of the protein matrix show the effect of various solvent extractions. Free lipids were distributed throughout the protein matrix rather than in distinct bodies. Bound lipids, however, appeared in small inclusions throughout the protein matrix and are assumed to be remnants of cytoplasmic structures occurring in endosperm cells at maturity. No difference was observed in the protein matrix which would suggest that it could be separated into wedge protein and a lipid-rich, fibrous, adhering protein fraction.

Flour lipids contain a mixture of more than 20 components (1) which can be classified into two main groups: "free" lipids or the nonpolar group, and "bound" lipids or the polar group. Wheat flour milled from hard wheats normally has 1–2% lipid, of which 60% is free and 40% bound (2). These lipids occur almost entirely in the protein matrix of wheat where their concentration is around 10%.

Work on the distribution of lipid in the protein matrix was initiated by Hess (3). He concluded that there was a large concentration of bound lipids high in phospholipid content in the area surrounding the starch granules. He also differentiated between this lipid-rich protein, which had a fibrillar structure, and the matrix protein, which was amorphous.

The purpose of this investigation was to determine the distribution of free and bound lipids in the protein of wheat endosperm with an electron microscope. Thin-sectioning techniques were employed rather than the less definitive replica procedures.

MATERIALS AND METHODS

Flour from a hard red spring wheat, Thatcher variety, was air-classified, and a separate portion of the coarse residue fraction (the vitreous parts of the kernel) was extracted with one of each of the following solvents: acetone, butanol saturated with water, ethanol-methanol 1:1 v./v., ethanol-diethylether 1:1 v./v., and petroleum ether (pentane-hexane mixture: Skelly F).

The extraction procedure used was as follows: A 1-g. sample was put into a small bottle with 30 ml. of solvent at room temperature. The bottles were shaken for 1 hr. on a wrist-action shaker so that no particles were held on the sides of the bottles. The spent solvent was then replaced with fresh solvent, the bottles were shaken for another hour, and then the samples were left overnight in the solvent. Again the spent solvent was replaced with fresh and the bottles were shaken for an hour. The solvent was withdrawn and the extracted samples were placed on watch-glasses for air-drying.

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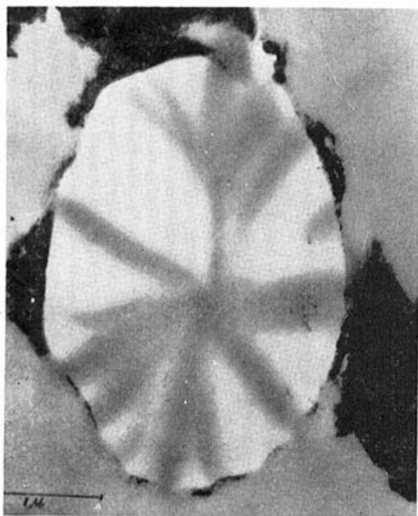
An unextracted flour sample plus the five samples extracted with different solvents were all fixed and embedded by the following procedure: Small amounts of sample were fixed in 2% osmium tetroxide at room temperature for 25 min., washed three times, and dehydrated for 48 hr. in cold ethanol (dry-ice temperature) with one change after 24 hr. The ethanol was then replaced with cold propylene oxide and gradually warmed to room temperature. Samples were then infiltrated with a 50:50 mixture of propylene oxide and Maraglas embedding resin consisting of 50 g. Maraglas epoxy resin 655, 8 g. Cardolite, 5 g. dibutylphthalate, and a few drops of benzyldimethylamine (4). After infiltration, the propylene oxide mixture was replaced with Maraglas embedding resin and the samples were evacuated to about 1.2 mm. Hg for a few minutes or until bubbling ceased. The resin was polymerized at 65°C. for 48 hr.

Thin sections were cut on a Porter-Blum ultramicrotome with glass knives and examined with an RCA EMU 3F electron microscope.

RESULTS

The association between a lipid layer and "adhering protein" has been described by Hess (5). In an attempt to clarify his concept, we examined particles from high-protein fractions of air-classified flour. Figure 1 shows



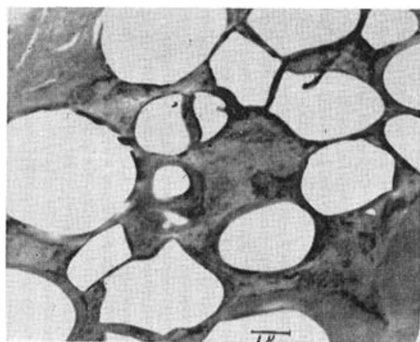


Fig. 1 (left). Starch granule with attached wedge protein stained heavily with osmic acid.

Fig. 2 (right). Control section of wheat endosperm. Unextracted sample of Thatcher wheat stained with osmic acid.

a thin section of a particle which consists of a single starch granule with some attached protein. The particle was purposely overstained so as to detect any thin layer of lipid or protein that might be attached to the outer surface of the starch granule. We did not find a continuous layer of protein around the starch granule. The process of milling or grinding caused a random separa-

tion of protein, as is evident in the figure. These small patches of protein on the surface of starch granules, seen when flour is viewed under a light microscope (6; see Fig. 2, p. 44), are part of the matrix protein. The adhering protein that Hess describes has an average thickness of 0.217 μ and can be seen only under an electron microscope (7). From examination of these particles, we observe no differentiation between so-called wedge protein and adhering protein.

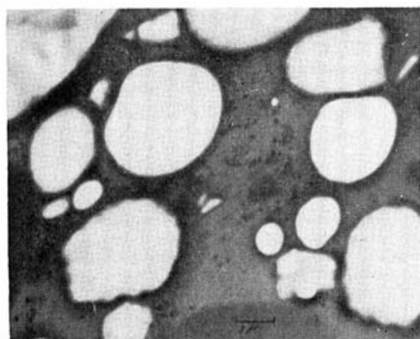
A typical section of unextracted wheat endosperm is shown in Fig. 2. The importance of this control section is the demonstration of inclusions in the protein matrix. These dark-staining areas presumably are rich in unsaturated lipids, because osmic acid reacts primarily with the unsaturated double bond in lipids (8). These inclusions are probably remnants of plastid membranes and cytoplasmic bodies present at the time of kernel maturation. In general, these inclusions are randomly distributed throughout the protein matrix.

The clear areas in this electron micrograph and subsequent ones are voids left by unfixed starch. Starch disintegrates in the process of cutting thin sections on a liquid trough.

A more troublesome artifact is the structureless band around some of these voids. This band may be lighter or darker than the adjoining protein matrix. Investigation shows that this band is the result of unfixed starch granules shrinking within a fixed protein matrix and the intervening space being infiltrated with embedding resin. In Fig. 1, the starch granule is not completely enclosed in protein; consequently, the artifact does not occur.

The effect of extraction with different solvents is illustrated by Figs. 3–7. The various solvents were chosen to include polar and nonpolar solvents, and mixtures of solvents that have been used by various workers to extract flour lipids.

Petroleum ether, a typical nonpolar solvent, was used to extract the free lipids from flour. Figure 3 shows a section of the extracted flour particle. Although the free lipid represents more than half the total lipid in flour, the



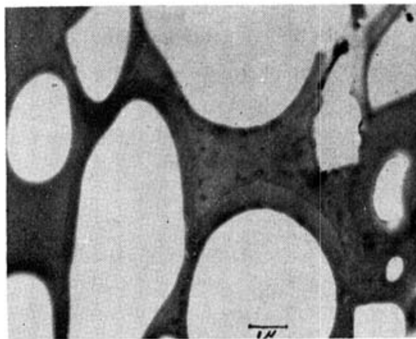


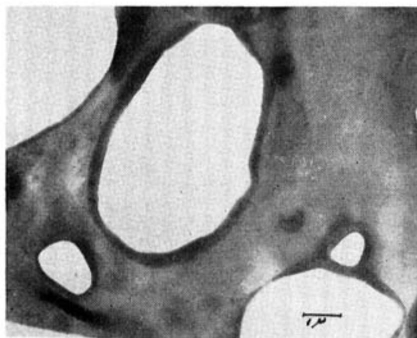
Fig. 3 (left). Flour particle extracted with petroleum ether and stained with osmic acid.

Fig. 4 (right). Flour particle extracted with acetone and stained with osmic acid.

general appearance of the protein matrix is similar to that of the control. The dark inclusions are easily noted and indicate sites of bound lipids. The only difference noticed is somewhat less contrast in staining of the protein matrix. The free lipids are, therefore, assumed to be distributed rather uniformly throughout the protein matrix.

Figure 4 reveals the effects of acetone extraction on wheat endosperm. The protein matrix is spotted with inclusions similar to those seen in the previous electron micrographs. Since acetone is a poor solvent for phospholipids, these inclusions are presumed to be rich in phospholipid material. A concentration of phospholipid is not evident at the starch protein interface.

Figure 5 represents butanol-extracted wheat endosperm. Only a few dark



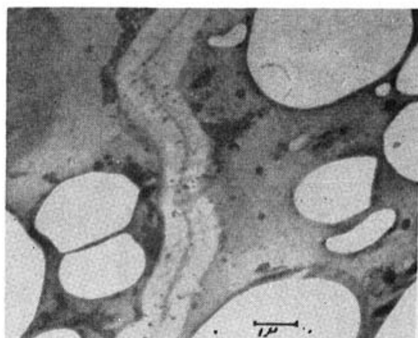


Fig. 5 (left). Flour particle extracted with butanol saturated with water and stained with osmic acid.

Fig. 6 (right). Flour particle extracted with methanol-ethanol 1:1 and stained with osmic acid.

areas are visible and they have less contrast than in the previous pictures. These results are expected, because butanol extracts more lipid than any other solvent.

Figure 6 shows wheat endosperm after extraction with the mixed solvent, methanol-ethanol. This mixture was used by Hess (3) to extract the phospholipid fraction from adhering protein. The center of the picture shows a pair of endosperm cell walls with the middle lamella in the center of the double wall. Some stained areas are seen near the cell wall, as well as a few inclusions in the protein matrix. The extraction with this mixed solvent did not appear to be as complete as with the butanol-water solvent.

Methanol-ether was one of the best solvents for extracting wheat lipids, as judged by the lack of contrast in Fig. 7. A few inclusions are visible, but they are of low contrast; the protein matrix has a fairly uniform density.

An identical extraction study was made on a soft wheat, and the results were similar to those on the hard wheat. The only tangible difference appeared to be a decrease in the density of the stained inclusions after polar solvent extractions; this difference indicates more complete extraction of bound lipid in soft wheat as compared with hard wheat.

We have demonstrated with various lipid solvents the solubility of lipids present in the protein matrix. Electron micrographs show the distinction be-

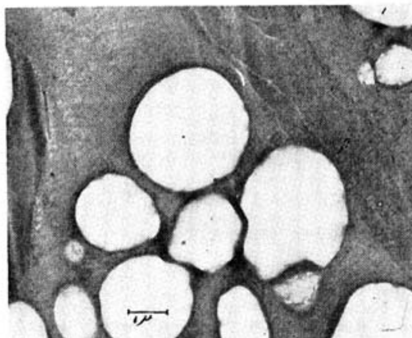


Fig. 7. Flour particle extracted with methanol-ether 1:1 and stained with osmic acid.

tween "free" and "bound" lipids which other workers have demonstrated by mass extraction methods. The free lipids were not seen as distinct bodies; instead, they were distributed throughout the protein matrix. The bound lipids, however, were found in small inclusions throughout the protein and are believed to be remnants of cytoplasmic structures present in the endosperm cells at the time of maturation.

DISCUSSION

Some controversy and confusion exist concerning the physical makeup of wheat protein. Hess introduced the terms "wedge" protein for the amorphous protein which fills the space between starch granules, and "adhering" protein for the protein which remains on starch granules after extensive ball-milling (3). He concluded from his work that the adhering protein had three to four times more bound lipid than the wedge protein and existed as a fibrillar network around starch granules. Other workers (9) who studied air-classified flour fractions reported that the percentage of bound lipids was highest for the low-protein fractions. They suggested that the lipid might be associated with a nonprotein component. Wrigley (10), however, found no evidence that the protein in air-classified fine fractions, which are high in protein, differed from that in the coarse low-protein fractions. Recent studies on the submicroscopic structure of wheat protein by Crozet *et al.* (11) show that under certain conditions a somewhat fibrous protein was observed adhering to both the starch granules and the matrix protein. Unlike Hess, however, they found no clear distinction between adhering protein and interstitial protein. In his studies of developing wheat endosperm, Buttrose (12) found no evidence of lipid bodies or lipid membranes as described by Hess and stated that Hess's results could be an artifact.

We conclude from our work on a hard red spring wheat and a soft wheat that a lipid-rich fibrous protein does not exist as a separate protein fraction having different properties from the matrix protein. We find, instead, a single, undifferentiated, amorphous protein with randomly dispersed inclusions extending up to the surface of starch granules.

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