

A Note on the Influence of Osmium Fixation on Wheat Flour Lipids Observation by Transmission and Scanning Electron Microscopy

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OsO₄ has been frequently used for electron microscopic fixation of proteins, lipids, and lipoproteins of wheat grain. It is known to oxidize unsaturated fatty acids and create diols on the site of ethylenic double bonds. It may also involve the formation of intermolecular linkages by breaking these double bonds and by forming an addition compound. This fixation increases the lipids contrast and makes them observable by electron microscopy.

In the protein bodies of endosperm cells Buttrose (1) and Morton et al. (2) observed unidentified areas after osmium fixation. In wheat flour or endosperm fragments many authors found osmiophilic globules that they identified as lipid inclusions (3-6). These osmiophilic granules are also present in the gluten lipoprotein compounds (5,6), in dough, and in the insoluble fraction in 4M urea of glutenin (7). However Hess (8), using a replica surface technique, indicated that in flour a layer of not-directly-observable lipids will surround the starch granules more or less uniformly. In the same way, observations by Aranyi and Hawrylewicz (9) and Evers (10) of flour surface using scanning electron microscopy did not show the presence of lipids zones or droplets; Aranyi and Hawrylewicz (9) only indicated a structural modification of proteins after *n*-butanol delipidation.

We have attempted to resolve this problem to see whether OsO₄ could aggregate unsaturated lipids observable in wheat flour, particularly on the starch-granule surface.

MATERIALS AND METHODS

Experiments were carried out on a flour obtained from a soft French wheat variety. For defatting, flour was extracted with pentane at 36°C. The lipids extracted represent 1.04% of the flour and are essentially "free" flour lipids.

OsO₄ fixation was made either by a 1% solution in acetate veronal buffer, at pH 7 for 30 min. at room temperature, or by dry OsO₄ vapors for 7 to 12 hr. at the same temperature.

For transmission electron microscopy the samples were dehydrated by ethanol and embedded in methacrylate before sectioning. For scanning observation they were coated with a fine layer of gold, then observed with a Jeol JSMU₃ electron microscope.

RESULTS

On a wheat flour section (Fig. 1, A) observed by transmission electron microscopy, there are osmiophilic globules at the starch-granule surface and at the periphery of protein fragments. The diameter of the globules ranges from 0.1 to 0.5 μ , sometimes greater. On a section from defatted wheat flour (Fig. 1, B), on the other hand, there are very few osmiophilic globules. The microscopic

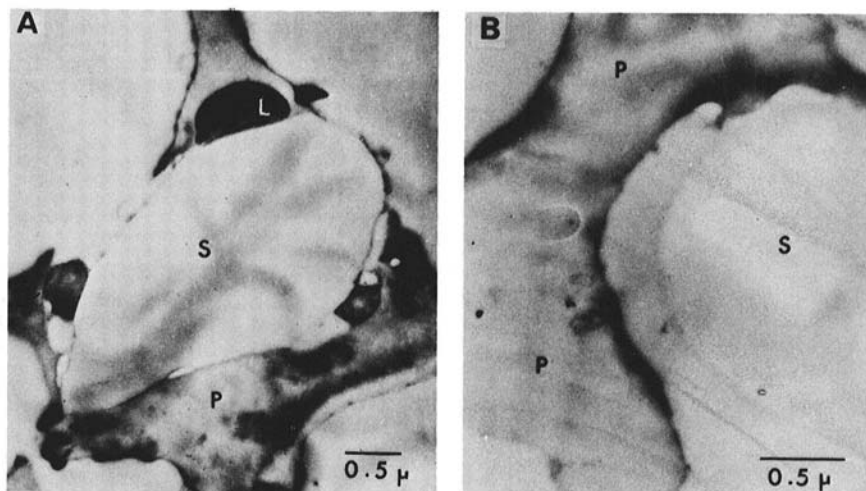


Fig. 1. Transmission electron microscopy of A, not-defatted flour, and B, pentane-defatted flour, OsO_4 fixation. S = starch granules, P = protein fragments, L = lipid globules.

observations seem to prove that osmiophilic globules would be essentially formed by the "free" lipids of flour extractable by pentane.¹

When untreated flour is observed under a scanning electron microscope (Fig. 2, A) one can only distinguish starch granules and proteins or lipoproteins fragments more or less adhering to the starch granules without any globular structure. On the other hand, in flour fixed by OsO_4 , we observe numerous globules on the starch-granule surface and on protein fragments (Fig. 2, B). On account of their size and place, these globules can be identified with those observed on sections by transmission electron microscopy. Elsewhere in the samples of defatted flour fixed by OsO_4 these globules are not visible (Fig. 2, C). This implies the lipid nature of these globules. We also observe defatted flour without osmium fixation (Fig. 2, D). In all pentane-defatted flour samples, we only notice a less compact protein structure than in normal flour and more fibrillar structures on the starch-granule surface.

Thus these observations suggest that fixation with osmium solution gives a globular aggregation of "free" flour lipids. The treatment by buffer solution without OsO_4 involves the formation of few globules. Moreover, in flour fixed by dry OsO_4 vapors (Fig. 3, A), there is an aggregation of lipids but different from the regular form of globules. At the starch-granule surface one can see globules and also zones with a more irregular shape. The latter are more difficult to differentiate from starch-adhering proteins. This can be compared with observations made by transmission electron microscopy on flour sections fixed by OsO_4 vapors. In this case osmiophilic zones are not only globular but often show elongated shapes (Fig. 3, B). Indeed fixation by OsO_4 vapors involves an aggregation of unsaturated lipids of flour, but their assembling gives less regular shapes than in aqueous medium.

¹Unpublished results, presented at the Journées sur l'amélioration des blés tendres, Action concertée DGRST, Paris, 1968.

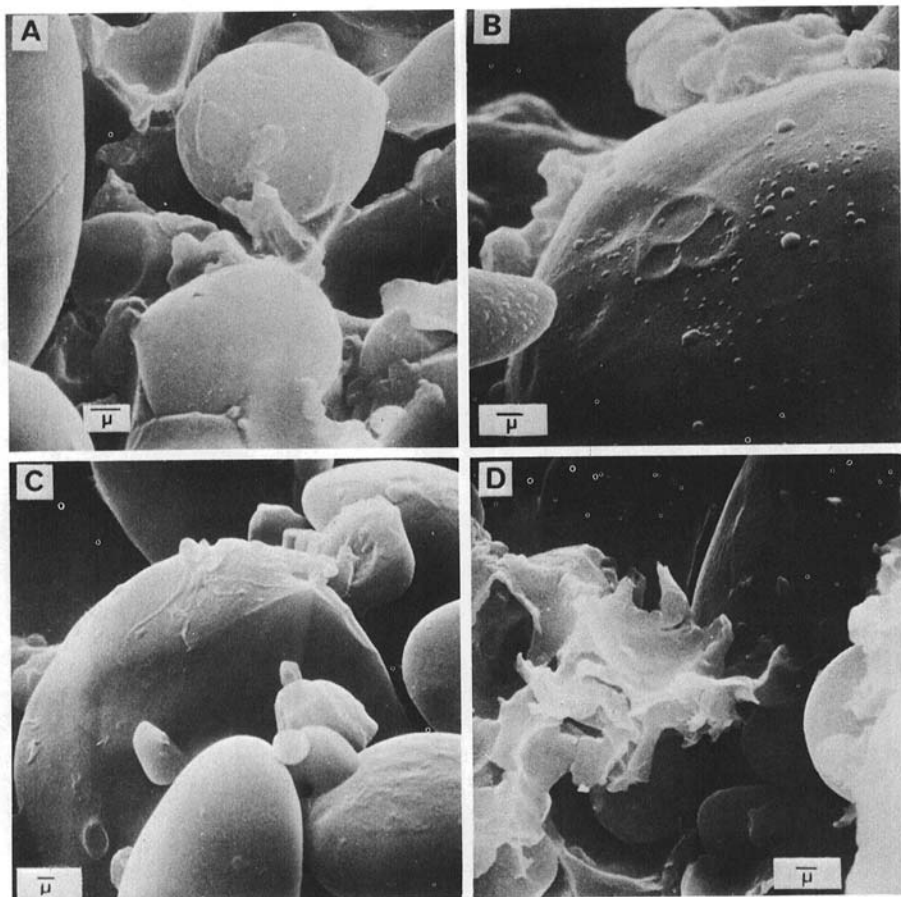


Fig. 2. Scanning electron microscopy of A, untreated flour; B, flour fixed by OsO_4 solution; C, defatted flour fixed by OsO_4 solution; and D, defatted flour without fixation.

DISCUSSION

These results show that at least a fraction of pentane-extractable flour lipids is aggregated to form globules during osmium fixation. It is possible that some lipid migration could occur during their aggregation where intermolecular linkages can arise. The globules, observed under transmission electron microscopy after osmium fixation, described on flour are also present on endosperm fragments. The globules are frequently associated with fibrillar zones, probably of a lipoprotein nature, which would arise from remnants of amyloplasts and other cytoplasmic membranous structures. Further study is necessary to determine whether the osmiophilic globules observed in these structures are in fact artifacts. The problem of the outline of "free" flour lipids cannot be resolved only by osmium fixation; moreover, it seems that an aqueous treatment before inclusion might induce some globules on starch surface in flour

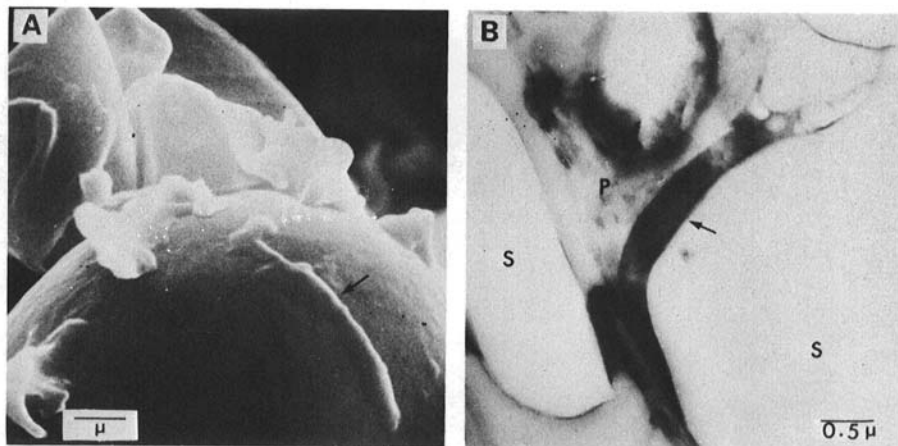


Fig. 3. Flour fixed by OsO_4 vapors observed by A, scanning electron microscopy, and B, transmission electron microscopy. Arrows indicate lipid aggregates.

particles. Further study will be necessary, however, for complete elucidation. The scanning technique seems to be useful for bringing more information for the purpose of a good interpretation.

The comparison of untreated defatted or not-defatted flours observed by scanning shows that proteins seem less compact after delipidation. Moreover, fibrillar structures adhering to the starch granules are visible. So it is possible to think that an important part of free lipids shifts more or less uniformly to the starch granule and protein interface; another part of "free" lipids, difficult to see under scanning microscopy, may take place in the protein matrix. This must be compared with the results obtained by Hess (8). Using a replica technique, this author previously indicated that a sheet of easily extractible lipids probably surrounds the starch granules, thus concealing fibrillar proteins and lipoproteins adhering to them.

In the case of lipoproteins fractions, obtained by lixiviation, where osmiophilic globules are present (5, 6) the scanning observations cannot give direct information. The problem remains to determine whether these globules occur during formation of gluten or under OsO_4 action.

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[Received May 9, 1973. Accepted August 21, 1973]