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MICROSTRUCTURE OF MEALY AND VITREOUS WHEAT ENDOSPERMS (*Triticum durum* L.)
WITH SPECIAL EMPHASIS ON LOCATION AND POLYMORPHIC BEHAVIOUR OF LIPIDS

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Abstract

Dark inclusions observed in osmiophilic zones, already described in mature wheat endosperm using transmission electron microscopy, were confirmed by freeze-fracture electron microscopy to be lipids. The polar lipids (glycolipids, phospholipids, free fatty acids, monoglycerides) were organized in a liquid crystalline phase. The reversed hexagonal or HII phase should be the main lattice which might arise from the transition of lipids present in membranous structures as a lamellar phase. This transition was caused by dehydration occurring during maturation. It is suggested that the water-dependent lamellar hexagonal phase transitions are of considerable importance in cereal food technology.

Introduction

Wheat lipids, though present at a low level (about 2% on a dry basis), are known to play an important role in cereal food technology such as breadmaking (MacRitchie and Gras, 1973; Chung et al., 1978).

The determination of location and structure of lipids in mature wheat endosperm is of considerable importance to explain their roles in the functional properties of wheat flour doughs and gluten.

In regard to its resolving power, scanning electron microscopy (SEM) is not sufficient to study lipid structures in the endosperm, so that transmission electron microscopy (TEM) is the best available method for this purpose.

In fact, TEM investigations of mature wheat endosperm are very limited because of major technical difficulties. Graham et al. (1963) and Jennings et al. (1963) did not examine any material more advanced in maturity than the 25th day after anthesis. To improve resin embedding, Simmonds (1972) infiltrated samples up to several weeks in glycol methacrylate and Parker (1980) infiltrated for two weeks with Spurr resin, endosperms aged up to 40th day after anthesis.

In TEM, lipids are viewed as osmiophilic inclusions and bilayer structures (Seckinger and Wolf, 1967; Simmonds, 1972; Bechtel et al., 1982) but long infiltration time and other treatments might form artifacts (Crozet, 1977).

Numerous works in the area of biomembrane research have shown that freeze-fracture electron microscopy (FFEM) is the best method to observe native lipid structures (Cullis and de Kruijff, 1979; Quinn and Williams, 1983) inasmuch as no chemical fixations are needed. Fretzdorff et al. (1982) have used FFEM with success to describe the ultrastructures present in wheat flour, dough, gluten and bread.

In the work reported here, FFEM was used to investigate for the first time lipid structures in mealy and vitreous endosperms. In order to compare our results with previous works, the organization of storage components was also determined by SEM and TEM. The latter method was improved in regard to the infiltration time.

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Key words: Freeze-fracture, lamellar hexagonal phase transition, lipids, protein matrix, scanning electron microscopy, transmission electron microscopy, vitreosity, wheat.

Materials and Methods

Wheat sampling

Two wheat varieties (*Triticum durum* L.), Wad 6292 and Mondur 1091 cultivated in the south of France and harvested in 1982 were studied. Wad 6292 is a vitreous variety (95 % vitreosity), and Mondur 1091 a mealy variety (47 % vitreosity) as determined by the method of Mauzé et al. (1972) modified by Al-Saleh and Gallant (1985). Water content 10% (dry basis) was measured according to the procedure of Multon et al. (1980). Samples for protein and lipid analysis were conditioned to 16% water content and milled to approximately 65% extraction on a Brabender Junior FL 1/4 mill.

Scanning electron microscopy

Grains were fractured transversally, mounted on copper stubs, and coated with a layer of gold (40nm) before observation in a JEOL JSM-50 A at 20 keV, absorbed current being less than 10⁻¹³ mA.

Embedding procedure and sectioning for TEM

³ Endosperm pieces measuring approximately 0.3 mm³ were cut from mature wheat grains with a razor blade under a zoom stereomicroscope. Samples were fixed, at room temperature, in 6% and 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 1 h respectively. Then, they were post fixed for 1 h in the dark under low pressure (1 to 2 torrs) in 1% osmium tetroxide in 0.1 M cacodylate buffer. Samples were dehydrated in graded acetone series, transferred successively into pure propylene oxide, propylene oxide-Epon 812 (50 : 50), and finally into pure Epon 812 for infiltration. The infiltration lasted for 24 h. Samples were sectioned with a glass knife on a JEOL JUM 7 ultramicrotome. Silver sections were stained for 30 min in 2.8% uranyl acetate in 50% methanol at 48°C and for 4 min in lead citrate (pH 13). Sections were viewed in a JEOL 100S at 80 keV.

Freeze Fracture

³ Small samples of wheat endosperm (about 0.3 mm³) were soaked in 90% glycerol-water solution (v/v) for 12 h. Specimens were frozen in liquid nitrogen slush obtained by vacuum. Freeze fracturing was carried out with a Reichert cryo-fracture apparatus, at -150°C and at least 10⁻⁷ torr. Fractured samples were shadowed with platinum from a 45° angle and carbon from a 90° angle. The replicas were cleaned in 50% chromic acid overnight, washed in distilled water, picked up on 400 mesh copper grids and viewed in a JEOL 100S microscope at 80 keV.

Lipid analysis

Nonstarch lipids from 15.0 g flour were extracted twice with chloroform:methanol (2:1 v/v) and finally with cold water-saturated n-butanol (65:35 v/v) at room temperature. The crude extracts were washed according to the procedure described by Folch et al. (1957). Lipids were dried, weighed and separated by silicic acid column chromatography into neutral lipids, glycolipids and phospholipids by eluting with chloroform, acetone and methanol, respectively (Rouser et al., 1967). Neutral lipids were separated by analytical thin layer chromatography (TLC) on 0.2 mm thick silica gel G plates (Merck), and were

viewed by spraying with 3% copper acetate in 8% orthophosphoric acid and charred at 180°C. Spot intensities were recorded with a Vernon densitometer. Glycolipid and phospholipid classes were separated by high performance liquid chromatography (HPLC) into their individual lipid classes according to the method described by Marion et al. (1984).

Protein analysis

Flour proteins were fractionated by Osborne's procedure into albumins and globulins, which were soluble in salt water (0.04 M NaCl); gliadin, soluble in 70% ethanol; and glutenin, the alcohol insoluble component. Protein contents were determined by the Kjeldahl method using a factor N x 5.7.

Results and Discussion

Organization of storage components in vitreous and mealy mature endosperms

Scanning electron micrograph of vitreous endosperm showed a continuous protein matrix covering the starch granules and giving a coherent physical structure to the endosperm (Fig. 1, arrowheads). On the contrary, the mealy endosperm exhibited a fragmented protein matrix with numerous air spaces (Fig. 2 arrowheads). These observations were confirmed by TEM of vitreous (Fig. 3) and mealy endosperm (Fig. 4). Especially in the case of mealy endosperm, large spaces were observed between starch granules and proteins (Fig. 4). Thus, vitreous endosperm was characterized by the compactness of the main components (starch, protein) which resulted from the continuity of the protein network. Our results were in accordance with those obtained with SEM by Stenvert and Kingswood (1977).

Figure 1. SEM of vitreous endosperm (Wad 6292), showing continuous protein matrix. Pn: protein matrix; S: starch.

Figure 2. SEM of mealy endosperm (Mondur 1091), showing fragmented protein matrix. Cw: cell wall; Pf: fragmented protein; S: starch.

Figure 3. TEM of vitreous endosperm (Wad 6292), showing continuous protein network and osmiophilic zones. Cw: cell wall; Oz: osmiophilic zone; Pn: protein network; S: starch.

Figure 4. TEM of mealy endosperm (Mondur 1091), showing fragmented protein network and osmiophilic zones. Cw: cell wall; Oz: osmiophilic zone; Pn: protein network; S: starch.

Figure 5. TEM of vitreous endosperm (Wad 6292), showing vestige of endoplasmic reticulum. Cw: cell wall; Er: endoplasmic reticulum; Oz: osmiophilic zone; Pn: protein network.

Figure 6. TEM of vitreous endosperm (Wad 6292). Cw: cell wall; Oz: osmiophilic zone; Po: zone of polysomes; Pn: protein network.

Figure 7. TEM of vitreous endosperm (Wad 6292), showing stage of protein body formation. Pb: protein body; Er: endoplasmic reticulum.

Figure 8. TEM of vitreous endosperm (Wad 6292), showing protein bodies to which ribosomes were attached. Pb: protein body; R: ribosomes.

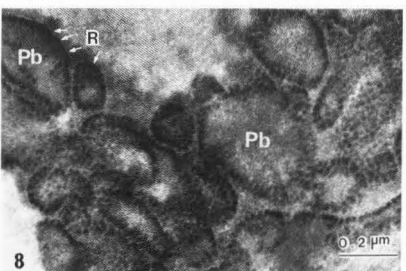
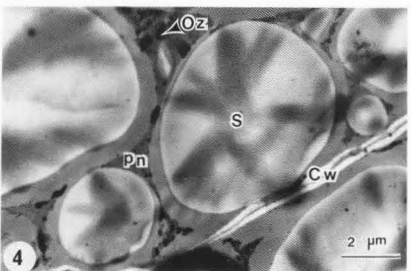
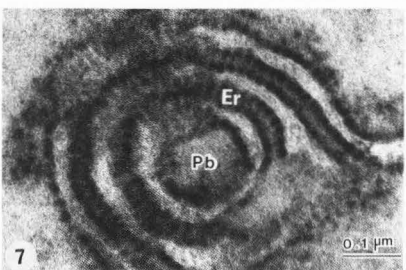
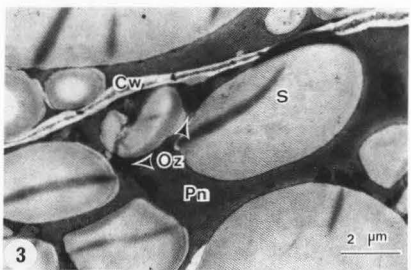
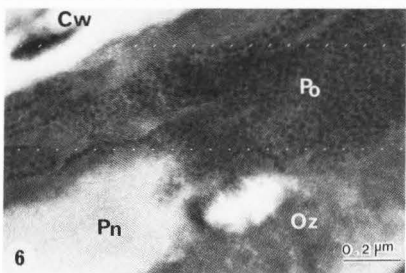
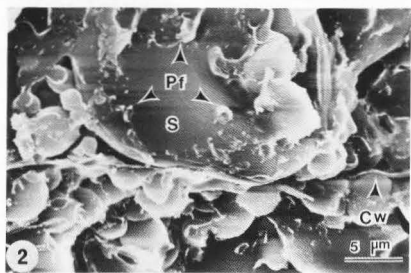
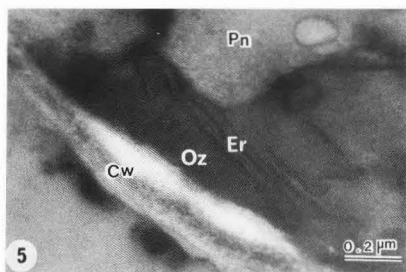
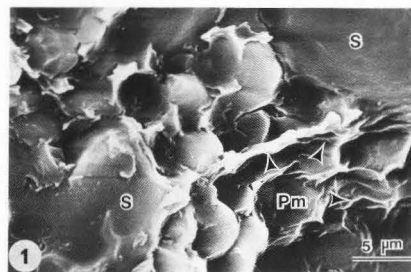


Table 1 : Composition and protein content (% dry matter) of the two varieties.

Variety	Albumins and globulins (% DM)	Gliadin (% DM)	Glutenin (% DM)	Total of protein fractions (% DM)	Protein content (% DM)
Wad 6292	2.50	6.20	5.11	13.80	15.00
Mondur 1091	2.05	3.20	4.30	9.55	10.00

Continuity of the protein matrix should be related to protein content; vitreous endosperm having a 15% protein content, whereas mealy endosperm has only 10% protein (Table 1). This relation has been already suggested by Stenvert and Kingswood (1977). The higher protein content in vitreous endosperm was mainly due to a higher gliadin content than in the mealy variety. Our protein compositions were quite different from those obtained by Nierle and Elbaya (1978) using other vitreous and mealy varieties which make it difficult to relate protein matrix continuity to protein composition.

Osmiophilic zones observed with TEM in the protein matrix were less numerous in the mealy endosperm than in the vitreous one. Rough endoplasmic reticulum (RER) and polysomes were observed in these osmiophilic zones (Figs. 5 and 6). For the first time, stages of protein body formation in mature vitreous wheat grains were highlighted such as: i) protein bodies enclosed in a spiral rough endoplasmic reticulum (Fig. 7), corresponding to cellular organelles similar to those already described by Bechtel and Juliano (1980) for the early stage of protein bodies formation in rice endosperm 7 days after anthesis; ii) protein bodies surrounded by a single membrane to which ribosomes were attached (Fig. 8); and iii) non-fused mature protein bodies (Fig. 9). The large number of polysomes and RER in vitreous endosperm suggested that an intensive protein biosynthetic activity had taken place during maturation, which might explain the higher protein content in the vitreous variety than in the mealy one. Non-fused protein bodies might be explained by grain dehydration which stopped coalescence of endosperm protein bodies. In addition to the cytoplasmic remnants, dark inclusions were observed (Figs. 3, 4 and 5) which have been suspected by many authors to be lipids (Seckinger and Wolf, 1967; Simmonds, 1972; Crozet et al., 1974; Crozet, 1977; Parker, 1980).

To our knowledge, no detailed work has been carried out on lipids in mature wheat endosperm, so that we have chosen to focus our investigations on the location and structure of these osmiophilic inclusions using FFEM.

Location and polymorphic behaviour of lipids in wheat endosperm

Photomicrographs of replicas obtained from freeze-fractured wheat endosperm showed different nonprotein structures located in large water areas or included in the protein matrix. Structures observed exhibited globular or tubular

shapes (Figs. 10, 11 and 12). According to work on biomembranes (Cullis and de Kruijff, 1979; Quinn and Williams, 1983), surfactants (Krog, 1981) and fats (Rigler et al., 1983; Timms, 1984) it was obvious that nonprotein inclusions were formed by lipids. The structures composed of stacked tubes, the mean diameter of which was 6 nm (Fig. 12), were in accordance with those of amphiphilic lipids (glycolipids, phospholipids, free fatty acids, monoglycerides) organized in a hexagonal liquid-crystalline phase (Deamer et al., 1970; Van Venetie and Verkley, 1981; Borovjagin et al., 1982). According to the work of Carlson et al. (1978) on the phase equilibria of extracted wheat flour lipids, this hexagonal structure was of reversed type (HII). In these structures a water channel was contained in each tube, with lipid arranged around this channel so that the polar head groups surrounded the water and the fatty backbone extended radially from the tube axis. The globular structures exhibited different kinds of fractures. In most replicas, only smooth vesicles measuring 20 to 300 nm were observed (Fig. 13), but in some cases, vesicles

Figure 9. TEM of vitreous endosperm (Wad 6292), showing non fused protein body. Pb: protein body; Pn: protein network; S: starch.

Figure 10. Freeze fracture of vitreous endosperm (Wad 6292), showing lipid vesicles. Lv: lipid lamellar vesicle; Mlv: lipid multilamellar vesicle; V: lipid vesicle; S: starch; W: water.

Figure 11. Freeze fracture of vitreous endosperm (Wad 6292). H: lipid hexagonal phase; Pn: protein network; S: starch.

Figure 12. Freeze fracture of vitreous endosperm (Wad 6292). H: lipid hexagonal phase; T: tubes (arrows); V: lipid vesicle.

Figure 13. Freeze fracture of vitreous endosperm (Wad 6292). H: lipid hexagonal phase; S: starch; V: lipid vesicle.

Figure 14. Freeze fracture of mealy endosperm (Mondur 1091). Mlv: lipid multilamellar vesicle; S: starch; W: water.

Figure 15 a. Freeze fracture of mealy endosperm (Mondur 1091). Ga: lipid granular aggregate; Mlv: lipid multilamellar vesicle; V: lipid vesicle; W: water. b. Magnification of multilamellar vesicle of figure 15a.

Figure 16. Freeze fracture of mealy endosperm (Mondur 1091). Mlv: lipid multilamellar vesicle; W: water.

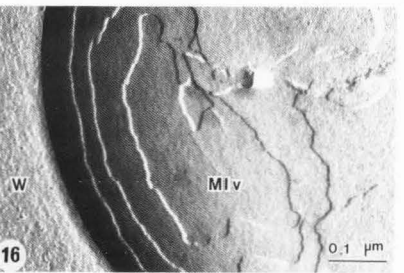
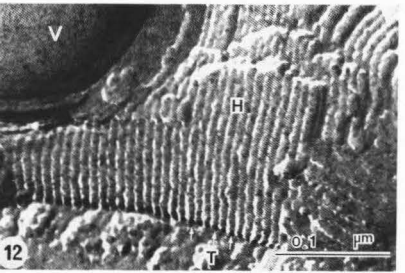
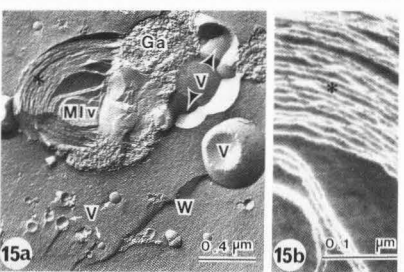
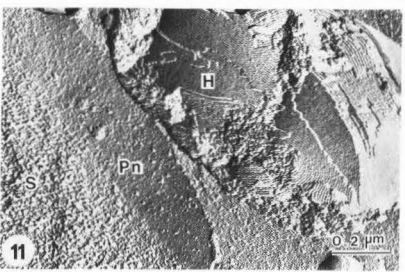
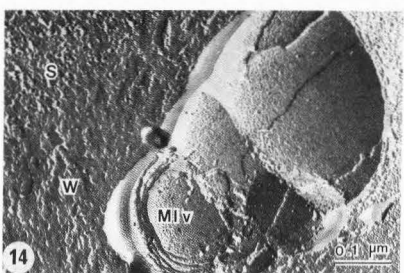
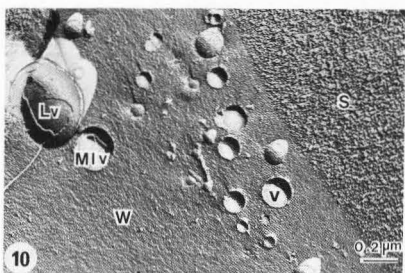
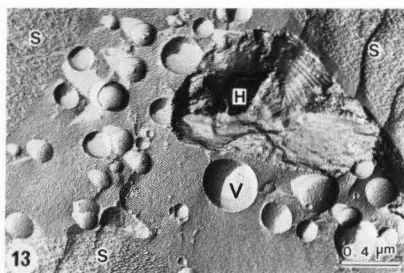
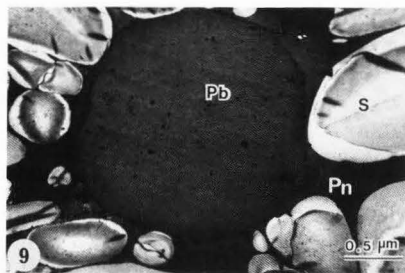


Table 2. Composition of lipids in vitreous and mealy endosperms (% dry basis and % total lipids in brackets)

Variety	Total lipids	Neutral lipids	Glycolipids	Phospholipids
Wad 6292	1.84	1.14 (61.9)	0.47 (25.4)	0.23 (12.7)
Mondur 1091	1.59	1.00 (63.4)	0.40 (25.4)	0.18 (11.2)

showed laminations (Fig. 14) and multilamellar structure was noted in fractured vesicles (Fig. 15 a,b). The lamellar repeat distance was about 5 nm (Fig. 16) which was similar to that obtained for polar lipids organized in a lamellar liquid-crystalline phase (Deamer et al., 1970; Shipley et al., 1973; Hui et al., 1981). The vesicles which did not show lamellar structure might be oil droplets stabilized by mono- or multilayers of polar lipids. These vesicles might also be composed of polar lipids arranged in unit or multilamellar structures.

It was noteworthy that vesicles were mainly observed in large water areas (Fig. 10), while tubes were located in protein matrix closely bound to starch granules, in a zone of low water content (Fig. 12). Moreover, the vitreous endosperm exhibited a structure more tubular than the mealy one. Mealy endosperm replicas, in the main, showed only lipid vesicular structures (Fig. 17 thick arrows). The composition of the main lipid classes did not highlight differences between vitreous and mealy varieties (Table 2). Moreover, TLC and HPLC chromatograms of mealy and vitreous endosperm lipids were quite similar. These results implied that lipid composition could not be responsible for the different polymorphic behavior of lipids in vitreous and mealy endosperm.

In fact, tubular structures were mainly observed in the central zone of replicas. Apparently the ratio of tubular to globular lipid structures decreased from the center to the outer area of the fracture face, so that only vesicles were observed in the outer area of vitreous endosperm replicas (Fig. 18). It is important to take into account the fact that 10% water was present in the cryoprotective medium which could diffuse into samples before cryofixation. Water diffusion was suggested by the fragmentation of the protein network near the outer area of the fracture face (Fig. 18, arrows) in comparison to the compactness of the protein network in the central zone (Fig. 11). Thus, the difference in the polymorphic behaviour of lipids in vitreous and mealy endosperms may be related to the difference in the speed of water diffusion. The low diffusibility of water in vitreous endosperm might be due to the compactness of the protein matrix.

We believe that the non-hydrated mealy endosperm exhibited many more tubular structures than were observed in the rehydrated one. Attempts to decrease water in the cryoprotective

medium did not allow us to obtain fractures of endosperms with the cryofracture device used in this work.

Thus the diffusion of water induced a transition of polar lipids from hexagonal to lamellar phases. Our conclusions are in accordance with the work of Carlson et al. (1978) which showed by X-ray diffraction that extracted wheat flour lipids exhibited a tubular structure below 15% water content and a lamellar structure between 15 and 50% water content.

The transition from hexagonal to lamellar phase has been studied by Vail and Stollery (1979). Many of their freeze-fracture photographs concerning the intermediate steps of transition were quite similar to what was observed in vitreous and mealy replicas, especially the granular aggregates surrounded by vesicles (Fig. 19). They suggested that the granular aggregates corresponding to an intermediate phase in the hexagonal to lamellar transition, were invaginations in lamellae, fusion of which produced the unilamellar vesicles covering the aggregates (Fig. 19). An association between a multilamellar vesicle, granular structure and smooth vesicles, which was not observed by Vail and Stollery (1979) during lipid phase transition, could be noted (Fig. 15). Vesicles could be released into the aqueous phase from the hexagonal structure observed in both vitreous (Fig. 20) and mealy endosperm (Fig. 21). In some cases, all hexagonal to lamellar transition steps were observed (Fig. 22).

Thus, the dehydration and rehydration of wheat endosperm could produce morphological changes in lipids by inducing lamellar-hexagonal phase transitions. Such events were recently shown in model membrane systems (Crowe and Crowe, 1982; Gordon-Kamm and Steponkus, 1984). It was obvious that similar transitions occurred during certain physiological or technological processes. Water loss during maturation, in particular, might induce a lamellar to hexagonal phase transition, as suggested in lettuce seed (Toivio-Kinnunen and Stushoff, 1981) whilst a hexagonal to lamellar phase transition might occur as seeds are soaked in water for germination. The lamellar structures observed by Simmonds (1972) in mature endosperm and by Bechtel et al. (1982) in endosperm, 28 days after anthesis, might arise from the hexagonal phases during the fixation steps carried out in an aqueous medium.

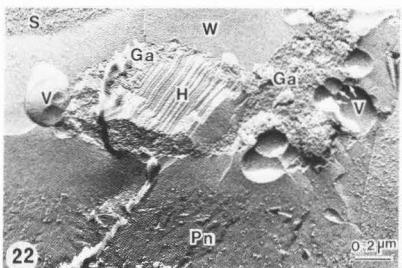
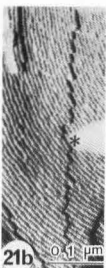
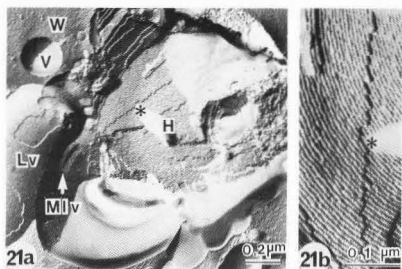
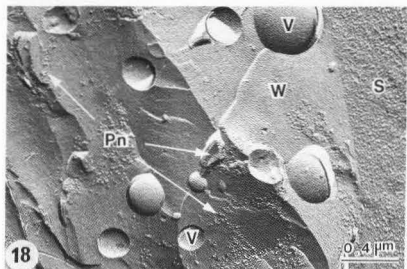
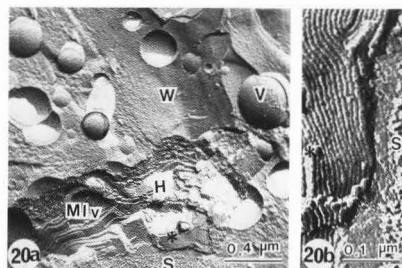
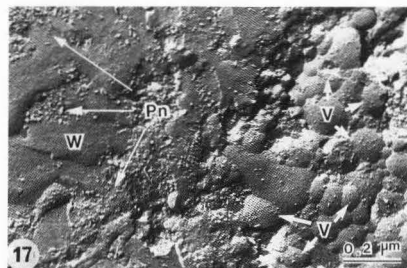


Figure 17. Freeze fracture of mealy endosperm (Mondur T091). Pn: protein network; V: lipid vesicles; W: water.

Figure 18. Freeze fracture of vitreous endosperm (Wad 6292), showing fragmented protein network. Pn: protein network; S: starch; V: lipid vesicle; W: water.

Figure 19. Freeze fracture of mealy endosperm (Mondur T091). Ga: lipid granular aggregate; V: lipid vesicle; W: water.

Figure 20 a. Freeze fracture of vitreous endosperm (Wad 6292). H: hexagonal phase; Mlv: multi-lamellar phase; S: starch; V: lipid vesicle; W: water. b. Magnification of hexagonal phase of figure a.

Figure 21 a. Freeze fracture of mealy endosperm (Mondur T091). H: hexagonal phase; Lv: lipid lamellar phase; Mlv: lipid multilamellar phase; V: lipid vesicle; W: water. b. Magnification of hexagonal phase of figure a.

Figure 22. Freeze fracture of vitreous endosperm (Wad 6292), showing lipid phase transition. Ga: lipid granular aggregate; H: hexagonal phase; Pn: protein network; S: starch; V: lipid vesicle; W: water.

Furthermore, such phase transitions might occur during dough development and in other steps of breadmaking technology. Kinetics of these transitions during dehydration or rehydration would bring new ideas on the role of lipids in physiological and technological processes.

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References

- Al Saleh A, Gallant DJ. (1985). Rheological and ultrastructural studies of wheat kernel behaviour under compression as a function of water content. *Food Microstruc.* **4**, 199-211.
- Bechtel DB, Gaines RL, Pomeranz Y. (1982). Protein secretion in wheat endosperm: Formation of the matrix protein. *Cereal Chem.* **59**, 336-343.
- Bechtel DB, Juliano BO. (1980). Formation of protein bodies in the starchy endosperm of rice (*Oryza sativa* L.): A re-investigation. *Ann. Bot.* **45**, 503-509.
- Borovjagin VL, Vergara JA, Mc Intosh TJ. (1982). Morphology of the intermediate stages in the lamellar to hexagonal lipid phase transition. *J. Membrane Biol.* **69**, 199-212.
- Carlson T, Larsson K, Miezis Y. (1978). Phase equilibria and structures in the aqueous system of wheat lipids. *Cereal Chem.* **55**, 168-179.
- Chung OK, Pomeranz Y, Finney KF. (1978). Wheat flour lipids in breadmaking. *Cereal Chem.* **55**, 598-618.
- Crowe LM, Crowe JH. (1982). Hydration-dependent hexagonal phase lipid in a biological membrane. *Arch. Biochem. Biophys.* **217**, 582-587.
- Crozet N. (1977). Ultrastructural changes in wheat-flour proteins during fixation and embedding. *Cereal Chem.* **54**, 1108-1114.
- Crozet N, Godon B, Petit L, Guilbot A. (1974). Submicroscopic structure of wheat flour and gluten lipoprotein components. *Cereal Chem.* **51**, 288-295.
- Cullis PR, De Kruijff B. (1979). Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta*, **559**, 399-420.
- Deamer DW, Leonard R, Tardieu A, Branton D. (1970). Lamellar and hexagonal lipid phases visualized by freeze-etching. *Biochim. Biophys. Acta*, **219**, 47-60.
- Folch J, Lees M, Sloane-Stanley GH. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.
- Fretzdorff B, Bechtel DB, Pomeranz Y. (1982). Freeze-fracture ultrastructure of wheat flour ingredients, dough and bread. *Cereal Chem.* **59**, 113-120.
- Gordon-Kamm WJ, Steponkus PL. (1984). Lamellar-to-hexagonal II phase transitions in the plasma membrane of isolated protoplasts after freeze-induced dehydration. *Proc. Natl. Acad. Sci. USA* **81**, 6373-6377.
- Graham JSD, Morton RK, Reason JK. (1963). Isolation and characterization of protein bodies from developing wheat endosperm. *Aust. J. Biol. Sci.* **16**, 375-383.
- Hui SW, Stewart TP, Yeagle PL, Albert AD. (1981). Bilayer to non-bilayer transition in mixtures of phosphatidylethanolamine and phosphatidylcholine: Implications for membrane properties. *Arch. Biochem. Biophys.* **207**, 227-240.
- Jennings AC, Morton RK, Palk BA. (1963). Cytological studies of protein bodies of developing wheat endosperm. *Aust. J. Biol. Sci.* **16**, 366-374.
- Krog N. (1981). Theoretical aspects of surfactants in relation to their use in breadmaking. *Cereal Chem.* **58**, 158-164.
- MacRitchie F, Gras PW. (1973). The role of flour lipids in breadmaking. *Cereal Chem.* **50**, 292-302.
- Marion D, Gandemer G, Douillard R. (1984). Separation of plant phosphoglycerides and galactosylglycerides by high performance liquid chromatography. In "Structure, function and metabolism of plant lipids". Siegenthaler PA and Eichenberger W (eds). Elsevier Science Publishers BV Amsterdam, 139-143.
- Mauzé C, Richard M, Scotti G. (1972). Contrôle de la qualité des blés; guide pratique (Wheat quality control; Practical guide-book). Institut Technique des Céréales et des Fourrages, Paris, France, 59-65.
- Multon JL, Bizot H, Martin G. (1980). Eau-humidités relatives (water-relative humidities). In "Techniques d'analyse et contrôle en industries agro-alimentaires" Lavoisier. Technique et Documentation (APRIA), **4**, 1-60.
- Nierle W, Elbaya AW. (1978). Relation between physical structure and some chemical constituents and milling performance of wheat kernels. *Chem. Mikrobiol. Technol. Lebensm.* **5**, 161-164.
- Parker ML. (1980). Protein body inclusions in developing wheat endosperm. *Ann. Bot.* **46**, 29-36.
- Quinn PJ, Williams WP. (1983). The structural role of lipids in photosynthetic membranes. *Biochim. Biophys. Acta*. **737**, 223-266.

Rigler MW, Roth IL, Kritchevsky D, Patton JS. (1983). The freeze-fracture ultrastructure of peanut oil and other natural and synthetic triacylglycerol droplets. *JAOCS*, 60, 291-298.

Rouser G, Kritchevsky G, Yamamoto A. (1967). Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. In "Lipid chromatographic analysis". Marinetti GV. (ed). E. Arnold Ltd, London, 1, 99-162.

Seckinger HL, Wolf MJ. (1967). Lipid distribution in the protein matrix of wheat endosperm as observed by electron microscopy. *Cereal Chem.* 44, 669-674.

Shibley GG, Green JP, Nichols BW. (1973). The phase behavior of monogalactosyl, digalactosyl, and sulphoquinovosyl diglycerides. *Biochem. Biophys. Acta.* 311, 531-544.

Simmonds DH. (1972). The ultrastructure of the mature wheat endosperm. *Cereal Chem.* 49, 212-222.

Stenvert NL, Kingswood K. (1977). The influence of the physical structure of the protein matrix on wheat hardness. *J. Sci. Food Agric.* 28, 11-19.

Timms RE. (1984). Phase behaviour of fats and their mixtures. *Prog. Lipid Res.* 23, 1-38.

Toivio-Kinnucan MA, Stushoff C. (1981). Lipid participation in intracellular freezing avoidance mechanism of lettuce seed (*Lactuca sativa* cultivar Grands Rapids). *Cryobiology*, 18, 72-78.

Vail WJ, Stollery JG. (1979). Phase changes of cardiolipin vesicles mediated by divalent cations. *Biochim. Biophys. Acta.* 551, 74-84.

Van Venetie R, Verkleij AJ. (1981). Analysis of the hexagonal II phase and its relation to lipidic particles and the lamellar phase: A freeze-fracture study. *Biochim. Biophys. Acta.* 645, 262-269.

Discussion with Reviewers

W.J. Wolf: Description of water content measurement is not clear. What is the significance of "10%" ? Could it be deleted ?

Authors: 10% is the water content of wheat samples before conditioning and is obtained by weighing wheat sample after heating at 133°C for 90 min. in an isothermal oven (French standard V 03-707).

W.J. Wolf: You state that the cryoprotectant contained 10% water but what was the water content of the wheat before and after exposure to the cryoprotectant ? I suppose that it would be difficult to determine the water content after exposure to the cryoprotectant, but it might be interesting to start with wheat samples of varying moisture contents and see whether there is a

moisture content that is in equilibrium with a 10% water content in the cryoprotectant. At equilibrium you should have a uniform structure from the exterior to the interior of the samples if your explanation of the effects of water diffusion is correct.

Authors: It would be effectively important to determine water and also glycerol content of wheat after exposure to the cryoprotectant but these determinations technically difficult in regard to the small size of endosperm samples used for freeze-fracture, should not allow to appreciate the water gradient from the exterior to the interior of the endosperm specimen.

We agree with you that equilibrium studies of water between cryoprotectant medium and wheat sample would allow to assess our hypothesis on the effects of water diffusion. However our first aim is to improve our freeze-fracture device in order to get replicas from noncryoprotected material. As a matter of fact it should be mentioned that glycerol is used only as a binding medium between sample and copper sheets to ensure a good fracture. In this low water sample cryoprotectant is not necessary because free water is not present and consequently, ice crystals cannot grow. Without use of cryoprotectant, it will be possible to assess our hypothesis on the effects of water diffusion and also to appreciate the ratio between lamellar and hexagonal structures in the native state.

D.D. Christianson: Is the total lipid extraction of the vitreous endosperm complete?

Authors: Our procedure is only able to extract nonstarch lipids so that total lipid extraction of wheat endosperm is not complete. However, concerning nonstarch lipids it is our own experience on freeze-dried gluten that this procedure allows extraction of at least 95% of total lipids on a fatty acid methyl ester basis.

D.D. Christianson: Are both these wheat varieties of breadmaking quality ?

Authors: These varieties are not used in breadmaking technology but only in pasta and semolina technology.

D.D. Christianson: What moisture level can be handled in the sample and still accomplish FFEM ?

Authors: Theoretically FFEM may be carried out on samples whatever the moisture content but some technical difficulties arise at low water content (5 to 15%). Above 20% cryoprotectant must be added to avoid ice crystal growth.

D.B. Bechte: Glycerol, being polar, will have a pronounced effect on endosperm lipids. How do you know that the structures you are looking at are not glycerol derived ?

Authors: Concerning the hexagonal structures they are mainly observed in zones in which water has not diffused and consequently in zones without glycerol. Moreover it is known that glycerol and polyol sugars stabilize lamellar structure in medium of low water activity (Crowe et al., 1984 *Biochim. Biophys. Acta.* 769, 141-150.) so that we think that hexagonal structures are not glycerol derived.

Concerning the transition from the hexagonal to the lamellar phase we obtained the same FFEM micrographs than those of Vaíl and Stollery (1979) who did not use glycerol. Moreover kinetics of this transition during hydration of wheat flour were recently carried out in our laboratory by one of us (D.Marion, personal communication) and show that, during the first hours, hexagonal structures disappear while granular aggregates and lamellar structure (mainly vesicles) appear. After 4 hours only vesicles are seen. In this experiment, glycerol was added only just before cryofixation so that we think that water was mainly responsible for this transition and not the glycerol.

D.B. Bechtel: How can you identify large water areas depicted in Figs. 10, 14, 15a, 16, 17, 18, 19, 20a, 21a and 22 as such when water content is 10% and no etching was conducted? I believe what you identified as water is in actuality storage protein matrix. In addition, the vesicles you describe are not true vesicles but protein matrix inclusions.

Authors: Water areas are identified by comparison with the replicas of the cryoprotective medium. The water areas are due to the diffusion of the water contained in the cryoprotectant leading to the disruption of the continuity between starch and protein matrix and between lipids and proteins. These water areas are not present in the native state at low water content (10%). During this water diffusion some vesicles embedded in the protein matrix have been excluded in these water areas. This phenomenon has been observed during hydration of wheat flour and extraction by handwashing of wheat gluten (D.Marion, to be published).

D.B. Bechtel: The most glaring problem with the interpretations is the assignment of the various classes (types) of lipid to various structures. For example, Ga: Lipid granular aggregate, Mlv: Lipid multilamellar vesicle, V: lipid vesicle. As these have not been isolated and characterized either by chemical and/or cytochemical methods, there can be no basis for such conclusions.

Authors: These lipid structures have been described by many authors in native and model membrane systems (see the review of Cullis and De Kruijff (1979) and of Quinn and Williams (1983); the original work of Gulik-Krzywicki et al. (1984) in: Surfactants in Solution (K.L. Mittal and B. Lidman, Eds), Plenum Press, New York, 1, 237-258).

D.B. Bechtel: Since only about 2% by weight is lipid in endosperm, and much of the lipids that is present is tied up on plasmalemma, amyloplast membranes, ER, etc., how can you expect to see so many lipid bodies (inclusions) at such a high frequency?

Authors: The main conclusion of our work is that during the final step of maturation, dehydration implies the transition of most residual membranes from a lamellar to a hexagonal structure and that during hydration of wheat endosperm during germination or for chemical fixation lamellar structure are regenerated mainly as vesicles. If we consider a vesicle with 1000 nm in diameter,

the partial specific volume of lipid molecules (0.98 cm³/g) and the density of the wheat grain (1.4 g/cm³), it is possible to calculate the number of vesicles by volume unit. In these conditions we found about 25,000 vesicles / mm³ of endosperm.

K. Larsson: The granular aggregates are related to the transition from the hexagonal to the lamellar phase. Recent work has shown that cubic phases with lipid bilayers forming an infinite periodic minimal surface are common between the hexagonal and lamellar phases. The morphology shown in fig. 19 is also consistent with that of such lipid-protein-water phases, as demonstrated by Gulik-Krzywicki et al. (1984) in: Surfactants in Solutions (K.L. Mittal and B. Lindman, Eds), Plenum Press, New York, 1, 237-258. Do the authors have additional EM information showing if the periodicity of this phase always is the same as in Fig.19 or if the repeat distance can vary?

Authors: The periodicity of this phase is almost the same in these granular aggregates and the repeat distance is comprised between 12 and 13 nm, value consistent with a cubic phase such as this one observed by Gulik-Krzywicki et al. (1984).

E.A. Davis: Do you believe that the functionality of certain flours may be affected by the participation of water in lipid phase changes in preference to hydrating the starch and wheat proteins of flour?

Authors: To our knowledge this problem has not yet received attention and will have to be considered in the future.