

Endosperm Structural Changes in Wheat During Drying of Maturing Caryopses

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ABSTRACT

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A transmission electron microscopic (TEM) study was conducted on maturing wheat to determine the sequences of events involved during the drying of preripe starchy endosperm. Field-grown hard red winter wheat Karl and soft red winter wheat Clark were harvested at 21 days after flowering (DAF) and air-dried in the spike at 20°C for 2, 4, 8, 12, 24, 48, and 96 hr and until completely air-dried (7 days). Fresh samples of Karl and Clark were also harvested and prepared immediately for microscopy. Both wheat cultivars underwent similar changes during drying. Few changes were observed during the first 8 hr of drying. By 12 hr after the start of drying, the rough endoplasmic reticulum (RER) was distended and circularized. Protein bodies were irregular in shape and small auto-

phagic vacuoles were found in the cytoplasm. An amorphous material was first observed in areas of the cytoplasm after 24 hr of drying. RER was typically associated with those regions. Endosperm tissue looked nearly mature after 48 hr of drying. Artificially induced senescence caused by harvesting wheat spikes prematurely caused the endosperm tissue to undergo a number of changes that resulted in the tissue looking normal when compared with wheat that was not prematurely harvested. This suggests that the wheat plant has great capacity to develop normally when subjected to environmental stresses. The methods used in this study can be used to investigate endosperm structural changes caused by adverse environmental stress.

The effects of environmental and genetic factors on wheat flour quality have been studied extensively (Lukow and McVetty 1991; Peterson et al 1992; Huebner et al 1997; Bergman et al 1998; Ames et al 1999). Although the genetic background of commercial wheats has been improved, environmental influences still continue to adversely affect wheat quality. Several studies on the effects of high temperature during grain filling and drying showed that harvested preripe wheat possessed flour quality traits superior to those of flour from wheat left to mature in the field (Finney 1954; Finney and Fryer 1957; Finney et al 1962). Finney (1954) found wheat that was harvested 10–14 days before ripe exhibited optimum loaf potential as well as excellent crumb grain. In addition, dough mixing requirement and mixing tolerance of flour from preripe wheat was generally superior but never less than dough from wheat harvested at field maturity. Maximum loaf volume potential corresponded developmentally to the time that storage protein bodies fused to form the protein matrix found in mature wheat endosperm (Bechtel et al 1982). Endosperm texture was not influenced by stage of development but was influenced by how the grain was dried (Bechtel et al 1996). Bechtel and Wilson (1997) studied the structure of wheat endosperm harvested at various stages preripe with freeze-dried or air-dried grain as it related to the formation of endosperm texture. The most pronounced change upon air drying of preripe samples was the disappearance of individual protein bodies and a corresponding conversion of the protein bodies and cytoplasm into a matrix-like material similar in appearance to that of storage protein matrix found in mature wheat endosperm.

The present work follows senescence of living starchy endosperm tissue caused by dehydration. An understanding of how cellular components degrade and interact during endosperm senescence may provide insight into how it is related to various quality traits important in the marketing of wheat.

MATERIALS AND METHODS

The hard red winter wheat Karl and soft red winter wheat Clark used in this study were field-grown near Manhattan, KS, USA. Wheat spikes were tagged at flowering and harvested at 21 days after flowering (DAF). The total maturation time from flowering to 14% moisture was 35 days and typical for the Manhattan, KS area. Entire wheat spikes with stems 25-cm long attached were collected at 21 DAF and allowed to air dry at 20°C in the laboratory for 0, 2, 4, 8, 12, 24, 48, and 96 hr, and for 7 days. Endosperm samples were prepared for microscopy by removing florets from the center of the spike, carefully dissecting caryopses from the glumes, and cutting the grain transversely into small pieces with a new razor blade. The endosperm samples were fixed in 2% (v/v) glutaraldehyde in 0.05M phosphate buffer, pH 7.2, at 4°C for 5 days. Samples were washed at least three times with buffer or until all odor of glutaraldehyde was removed, and then postfixed in 1% (w/v) osmium tetroxide in 0.05M phosphate buffer, pH 7.2, for 2 hr. Fixed tissue was dehydrated in a graded acetone series (30, 60, 90, 100%, v/v) and embedded in Spurr resin. Plastic sections (1 µm thick) were cut with glass knives, stained in a mixture of Toluidene Blue O (0.365 g) and Basic Fuchsin (0.135 g) dissolved in 50 mL of 30% (v/v) ethanol, mounted with a permanent mounting medium and a coverslip, and viewed with phase-contrast light microscopy (LM). The LM was used to select appropriate areas for transmission electron microscopy (TEM) study. Blocks of tissue were retrimmed with a razor blade and silver-colored ultra-thin sections (≈100 nm) were cut with a diamond knife on an ultramicrotome. The thin sections were stained with 2% aqueous uranyl acetate (w/v) and lead citrate, and micrographs were recorded as previously described (Bechtel and Pomeranz 1978).

RESULTS

No changes were observed among the samples of wheat that had been dried for 0, 2, 4, and 8 hr from those samples prepared from fresh caryopses. In addition, the hard and soft red winter wheats could not be distinguished from one another at the ultrastructural level. The descriptions of the structural changes during drying, therefore, were combined, and representative micrographs from each drying stage and wheat were used to describe the endosperm structure. Freshly fixed 21 DAF control subaleurone starchy endosperm cells of Karl and Clark were similar and exhibited cells in various stages of protein body fusion, signifying

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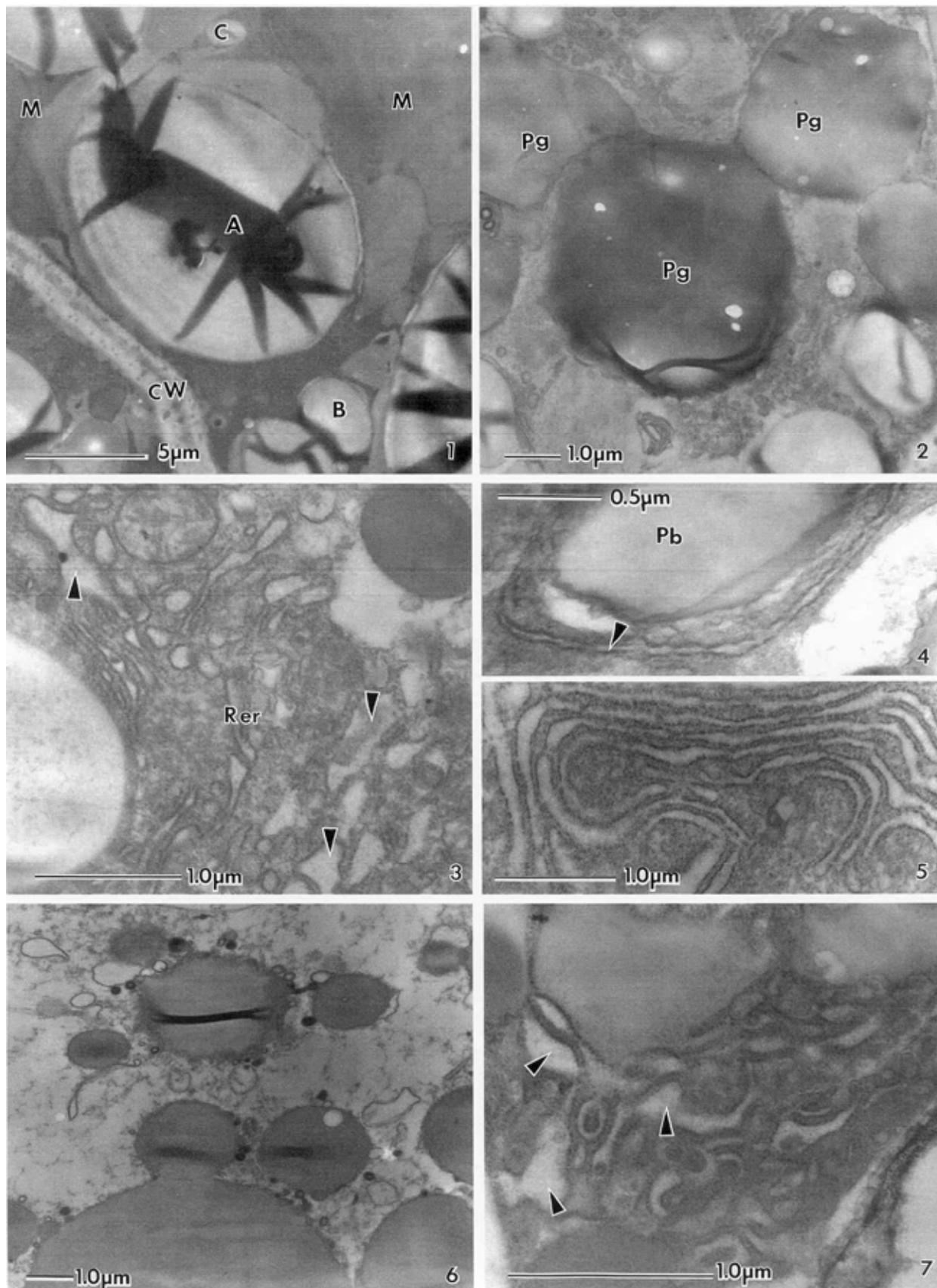


Fig. 1. Plate 1: Subaleurone starchy endosperm of freshly harvested Karl 21 DAF wheat showing type A, B, and C starch granules, cell wall (CW) with cytoplasm closely appressed to it and storage protein forming the matrix (M) (5,200 \times). Plate 2: Protein granules (Pg) within vacuole fusing to form the protein matrix in freshly harvested Karl subaleurone endosperm (9,600 \times). Plate 3: Starchy endosperm of freshly harvested Karl endosperm showing an unusually large amount of RER (Rer) that is distended where RER branches (arrows) (24,700 \times). Plate 4: Fresh Clark endosperm with long single cisterna of RER (arrow). Protein body (Pb) (36,500 \times). Plate 5: RER from 21 DAF Karl endosperm that had been dried for 8 hr. RER is distended along the entire cisterna of the RER (21,700 \times). Plate 6: Clark endosperm dried for 2 hr showing protein granules in the central vacuole fusing to form the incipient protein matrix (8,800 \times). Plate 7: Clark endosperm dried for 12 hr showing densely stained cytoplasm and distention of the RER (arrows) (35,400 \times).

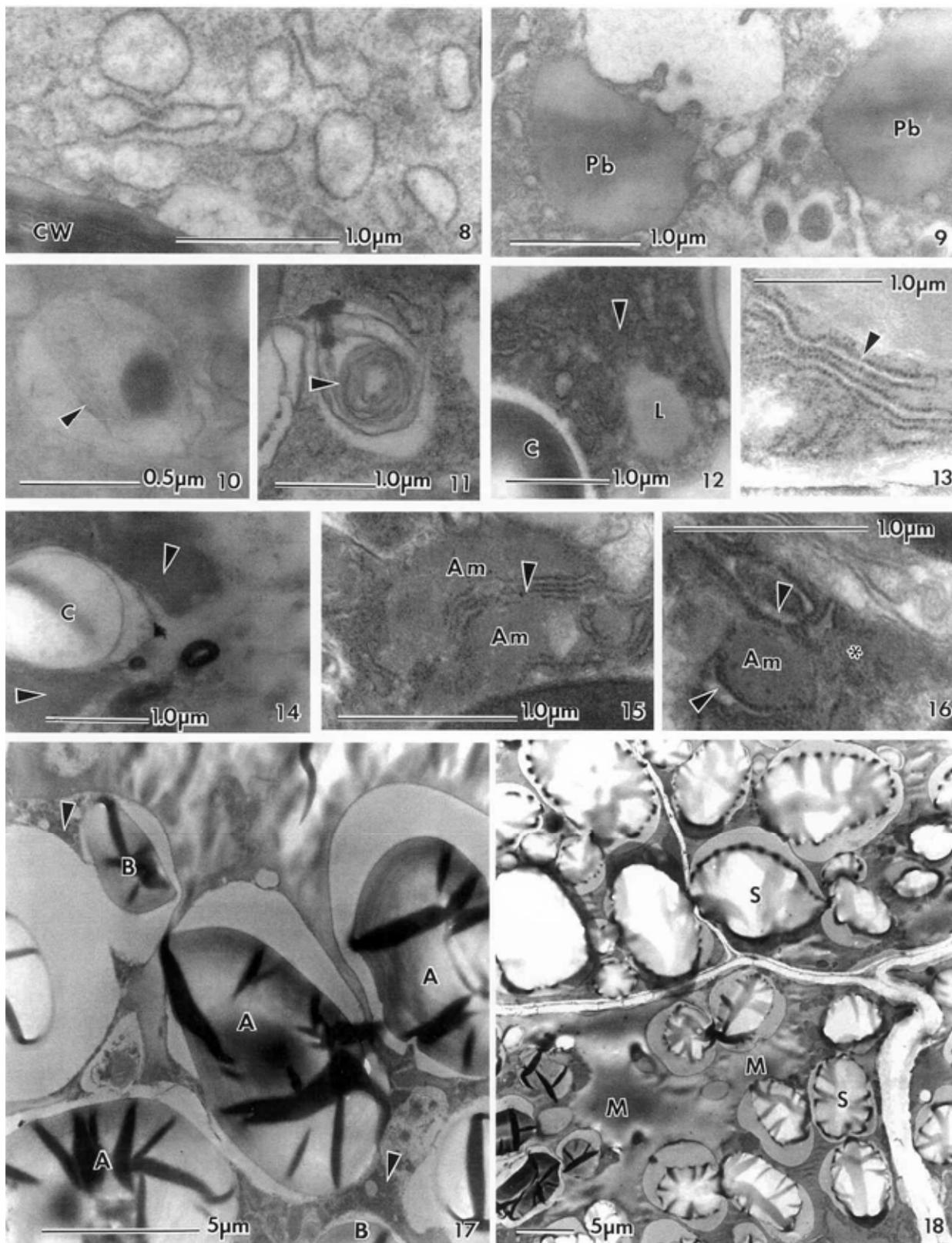


Fig. 2. Plate 8: Karl endosperm dried for 12 hr possessing circular RER cisternae near cell wall (CW) (28,900 \times). Plate 9: Clark endosperm dried 12 hr showing protein bodies with irregular surfaces (Pb) (22,200 \times). Plate 10: Partially digested cytoplasm (arrow) in autophagic vacuole in endosperm of Clark dried for 12 hr (37,800 \times). Plate 11: Lamellar whorl (arrow) in endosperm cell of Karl wheat dried for 12 hr (17,000 \times). Plate 12: Densely stained cytoplasm (arrow) in the endosperm of Karl dried for 24 hr. A small type C starch granule (C) and lipid droplet (L) are also present in the cytoplasm (16,900 \times). Plate 13: RER (arrow) found in a small region of cytoplasm of Clark endosperm dried for 24 hr (21,600 \times). Plate 14: Area of cytoplasm of Karl dried for 24 hr showing densely stained amorphous material (arrows) surrounding a small type C starch granule (17,500 \times). Plate 15: Amorphous material (Am) with interspersed RER (arrow) in Clark endosperm dried for 24 hr (30,600 \times). Plate 16: Amorphous material (Am) with interspersed RER (arrows) in Clark endosperm dried for 24 hr. Note free ribosomes (*) (31,700 \times). Plate 17: Low magnification view of Clark subaleurone endosperm dried for 24 hr showing matrix protein surrounding type A and B starch granules and isolated small regions of what was left of the cytoplasm in angular areas (arrows) (5,270 \times). Plate 18: Low magnification view of Karl subaleurone endosperm dried for 48 hr showing mature matrix protein (M) surrounding starch granules (S) (1,900 \times).

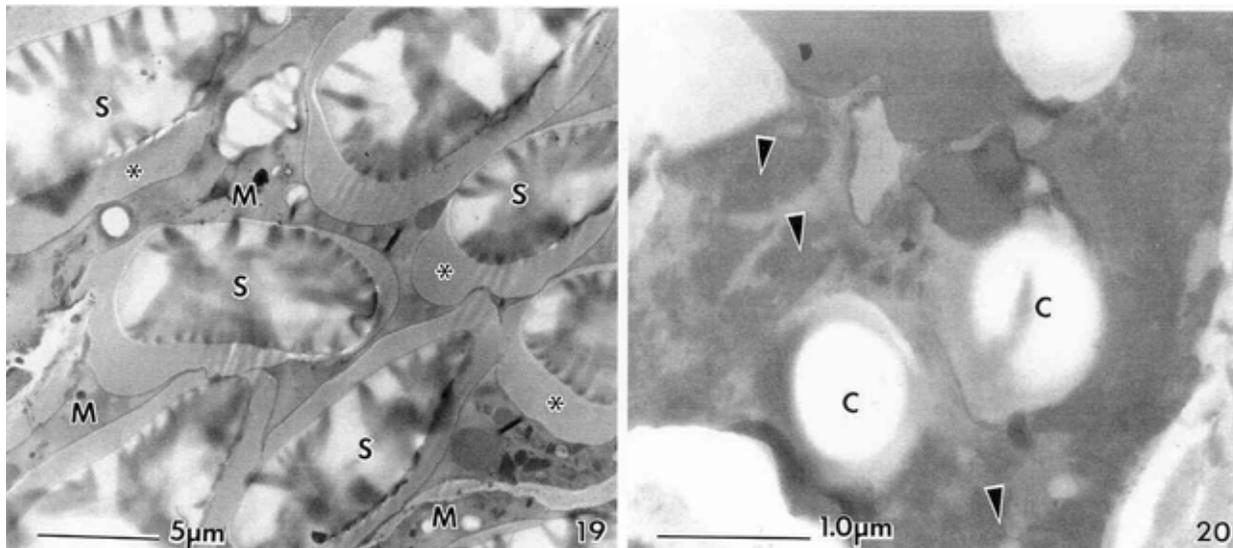


Fig. 3. Plate 19: Low magnification view of completely dry Karl subaleurone endosperm showing matrix protein (M) surrounding the starch granules (S). Spaces (*) between starch and matrix is an artifact caused by shrinkage of the starch granules during specimen preparation for electron microscopy (3,400 \times). Plate 20: Completely dry Karl endosperm showing densely stained amorphous material (arrows) surrounding type C starch granules (19,700 \times).

the formation of the storage protein matrix (Fig. 1, plates 1 and 2) (see Bechtel et al 1982 for a review of protein body terminology and matrix formation). Subaleurone cells possessed starch granules representing the three size classes present at maturity; large (type A), medium (type B), and small (type C) granules (Fig. 1, plate 1). The rough endoplasmic reticulum (RER) in 21 DAF endosperm consisted of long interconnected cisternal elements, of which some appeared slightly distended. However, the distended regions at this stage seemed to be at locations where the interconnections were sectioned obliquely (Fig. 1, plate 3). RER that occurred as individual cisterna rather than in groups of cisternae possessed uniform lumen that was not distended (Fig. 1, plate 4). The RER and storage proteins from samples dried 2, 4, and 8 hr were similar in appearance to that of the freshly fixed samples (Fig. 1, plates 5 and 6).

A number of changes were observed in the subaleurone endosperm dried for 8–12 hr. Starchy endosperm cells had cytoplasm that was denser and stained noticeably darker than previous stages of drying (Fig. 1, plate 7). The RER had more distentions than previous drying stages, with many cisternal elements giving the appearance that they were divided into circular elements rather than tubular elements (Fig. 1 plate 7; Fig. 2, plate 8). Protein bodies in the cytoplasm possessed an irregular surface in endosperm dried for 12 hr (Fig. 2, plate 9). Small autophagic vacuoles and lamellar whorls were observed in the cytoplasm (Fig. 2, plates 10 and 11).

Substantial ultrastructural changes were observed after caryopses were dried for 24 hr. The cytoplasm was compacted and densely stained (Fig. 2, plate 12). RER was found infrequently and, when observed, was located in isolated pockets of ribosome-rich cytoplasm between starch granules and protein matrix (Fig. 2, plate 13). Many areas of the cytoplasm possessed regions of an amorphous material that stained darker than the protein matrix (Fig. 2, plate 14). The amorphous material was typically associated with regions of the cytoplasm and was intimately associated with both the RER and the large numbers of free ribosomes (Fig. 2, plates 15 and 16). Most storage protein was in the form of matrix protein associated with small angular pockets of cytoplasm (Fig. 2, plate 17).

Ultrastructural differences were not observed among wheat endosperm samples dried from 48 hr to 7 days; therefore, these stages of drying are described interchangeably. Wheat caryopses dried for 48 hr contained endosperm cells that were converted to mature-appearing tissue with starch granules embedded in the

protein matrix and the cytoplasmic remnants relegated to the periphery of the cells (Fig. 2, plate 18). A-, B- and C-type starch granules were surrounded by the protein matrix and remnants of the cytoplasm (Fig. 3, plate 19). Cytoplasmic remnant regions contained densely stained amorphous material as well as other densely stained areas that were highly irregular (Fig. 3, plate 20).

DISCUSSION

The importance of the late stages of wheat development in determining the quality of bread was shown when wheat was harvested preripe at various stages and allowed to air dry in the spike. The dried wheat was then threshed, milled into flour, and baked into bread (Finney 1954; Finney et al 1962). Bread baked from wheat harvested 10–14 days preripe (DPR) (corresponding to 21–25 DAF [see Bechtel and Wilson 1997]) exhibited higher loaf volume potential and better crumb grain than wheat harvested earlier or wheat allowed to mature naturally in the field. In addition, flour milled from preripe caryopses showed similar or better mixing requirement and mixing tolerance than flour from mature wheat (Finney 1954). Bread baked from freshly squeezed preripe wheat endosperm also gave loaf volume potential superior to that of mature wheat (Finney 1954). Loaf volume potential was also affected by drying. In fact, Figs. 7 and 8 in Finney (1954), showed that freshly squeezed endosperm had a loaf volume potentials as high as 118% of the loaf volume expected from protein in the endosperm. Air-dried preripe grain had a loaf volume potential peak at \approx 110% for 14 DPR; it decreased to 100% at \approx 4 DPR. That data suggests that wheat from 21 DAF (14 DPR) to \approx 28 DAF (7 DPR) contains the maximum loaf volume potential available in the wheat caryopsis and the potential is reduced either by drying the wheat or allowing the wheat to mature fully.

The period where the maximum loaf volume potential occurred corresponded to the developmental stage where the individual wheat storage protein bodies fused with one another to form the protein matrix that is always present in mature endosperm cells (Bechtel et al 1982b). It is also one week before the wheat grain is termed physiologically mature (i.e., the point in grain development where there is no further gain in dry matter). The one-week period from 21 DAF (14 DPR) to 28 DAF (7 DPR) seems to be a critical timeframe in wheat development because it represents the only period where breadmaking properties are greater than in the mature grain. Understanding how and why breadmaking potential decreases

could allow breeding programs to focus on the genetic and biochemical reasons for the decreases and could lead to cultural and grain-handling practices that may minimize those decreases.

Although the overall time required for complete dehydration of the preripe wheat was the same (7 days) as for physiologically mature wheat (28 DAF) to dry (35 DAF), the major difference between the two samples may be that preripe 21 DAF endosperm was still alive when harvested, while the 28 DAF endosperm cells were dead (see Bechtel et al 1982). The dramatic cellular changes occurring within the preripe caryopsis and the subsequent conversion of live tissue into normal-appearing mature endosperm in a relatively short timeframe (48 hr) may account for the better bread-making characteristics of the preripe grain. The short timeframe may account for fewer enzymes interacting detrimentally with starch and gluten proteins. Autophagy, the process where portions of cells are partially digested during differentiation, is common during wheat endosperm development (Bechtel et al 1989). Surprisingly, the present study found little evidence of autophagy during endosperm dehydration. The lack of large numbers of autophagic vacuoles as endosperm tissue dries and dies clearly shows how tightly controlled the process of senescence is during wheat endosperm development.

LITERATURE CITED

- Ames, N. P., Clarke, J. M., Marchylo, B. A., Dexter, J. E., and Woods, S. M. 1999. Effect of environment and genotype on durum wheat gluten strength and pasta viscoelasticity. *Cereal Chem.* 76:582-586.
- Bechtel, D. B., and Pomeranz, Y. 1978. Ultrastructure of the mature ungerminated rice (*Oryza sativa*) caryopsis. The starchy endosperm. *Am. J. Bot.* 65:684-691.
- Bechtel, D. B., and Wilson, J. D. 1997. Ultrastructure of developing hard and soft red winter wheats after air- and freeze-drying and its relationship to endosperm texture. *Cereal Chem.* 74:235-241.
- Bechtel, D. B., Gaines, R. L., and Pomeranz, Y. 1982. Protein secretion in wheat endosperm—Formation of the matrix protein. *Cereal Chem.* 59:336-343.
- Bechtel, D. B., Wilson, J. D., and Martin, C. R. 1996. Determining endosperm texture of developing hard and soft red winter wheats dried by different methods using the single-kernel wheat characterization system. *Cereal Chem.* 73:567-570.
- Bechtel, D. B., Frend, A., Kaleikau, L. A., Wilson, J. D., and Shewry, P. R. 1989. Vacuole formation in the wheat starchy endosperm. *Food Microstruct.* 8:183-190.
- Bergman, C. J., Gualberto, D. G., Campbell, K. G., Sorrells, M. E., and Finney, P. L. 1998. Genotype and environment effects on wheat quality traits in a population derived from a soft by hard cross. *Cereal Chem.* 75:729-737.
- Finney, K. F. 1954. Contribution of the Hard Red Wheat Quality Laboratory to wheat quality research. *Trans. AACC* 12:127-142.
- Finney, K. F. 1957. Effect on loaf volume of high temperatures during the fruiting period of wheat. *Agron. J.* 50:28-34.
- Finney, K. F., Shogren, M. D., Hosney, R. C., Bolte, L. C., and Heyne, E. G. 1962. Chemical, physical, and baking properties of preripe wheat dried at various temperatures. *Agron. J.* 54:244-247.
- Huebner, F. R., Nelsen, T. C., Chung, O. K., and Bietz, J. A. 1997. Protein distributions among hard red winter wheat cultivars as related to environment and baking quality. *Cereal Chem.* 74:123-128.
- Lukow, O. M., and McVetty, P. B. E. 1991. Effect of cultivar and environment on quality characteristics of spring wheat. *Cereal Chem.* 68:597-601.

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