Endosperm Modification in Germinating Sorghum Grain

C. W. GLENNIE,[1] J. HARRIS,[2] and N. V. D. W. LIEBENBERG[3]

ABSTRACT

Several techniques were used to prepare germinated sorghum grains for both transmission and scanning electron microscopy and, hence, to study the patterns of modification that occurred in the endosperm during germination. Bird-resistant sorghum grain was germinated on moist filter paper at 28°C for 12 days, and samples were taken every day for microscopic examination. Endosperm modification began at the endosperm scutellum interface and subsequently moved into the floury endosperm, with slight modification of the peripheral endosperm. The horny endosperm was modified last. The matrix protein began to disappear first, and after it was disrupted, the starch granules and protein bodies were degraded simultaneously. The starch granules were modified by pitting rather than by surface erosion, and their interiors soon became hollow. Viewed from the outside, their form still remained. The aleurone cells did not appear to be active in enzyme production; rather the scutellum fulfilled this role. However, the aleurone cells were extensively modified during germination, and their mineral content was greatly reduced. The cell walls were the only part of the endosperm that appeared visually unchanged after germination; they retained their structure even after the endosperm was extensively modified and the cells had lost their contents.

Cereal Chem. 60(1):27–31

One of the objectives of malting of either sorghum or barley is to produce enzymes that are not present in the ungerminated grain. These enzymes are necessary for the breakdown of malt and adjuncts during brewing. Also, the malted grain must produce a product with desirable organoleptic qualities. Barley is a temperate crop, and barley malt is used to brew European-style beers. Hence, much information is available on barley modification during malting (Palmer 1980, Palmer and Bathgate 1976). However, in Africa, with its large, hot, semiarid regions, sorghum is the grain of preference.

Like barley, sorghum malting has received much attention. Novellie (1960a) described the soluble and insoluble amylases produced by different varieties of sorghum during malting. Insoluble amylases were found in malts from bird-resistant sorghum, but they were active in the insoluble state. Other studies revealed that 18–39% of the saccharifying activity of sorghum malt was due to β-amylase (Novellie 1960b). Both α- and β-amylases were isolated from sorghum malts and their properties described (Botes et al 1967a, 1967b).

Several attempts were recently made in hot climates to substitute malted sorghum for barley malt in the brewing of lager beer (Jayatissa et al 1980, Okafor and Anicke 1980). Although the lager produced from sorghum was acceptable, more research was recommended in order that the final product might be improved. In Africa, sorghum is generally used to make a more traditional brew (Novellie 1968) and, since this type of brewing has become industrialized, research has played an important role in its development (Daiber et al 1973). This study is an extension of previous research to try and improve our knowledge of the modification patterns that occur during the malting of sorghum grain.

Most of the sorghum grain grown in the United States is used as livestock feed; consequently, much research has been done on the grain. Scanning electron microscope (SEM) studies have established much of the structure and composition of the sorghum grain (Hoseney et al 1974, Seckinger and Wolf 1973, Sullins and Rooney 1975). This study explains some of the changes that occur in the grain during germination.

MATERIALS AND METHODS

Malting

Bird-resistant sorghum hybrid SSK52 was collected from the 1980 crop. The grain was surface-sterilized with 0.2% Westcodyne and steeped for 6 hr (the water was changed after 3 hr). Germination was accomplished on moist filter paper in petri dishes that were 9 cm in diameter (50 grains in each) at 28° C in a Forma Scientific hydro-jac CO_2 dual water-jacketed incubator. Each day, one dish of seedlings was taken, the root and shoot length measured, and the seedlings with root and shoot length nearest the average selected for examination.

Electron Microscopy

Material for transmission electron microscopy (TEM) was fixed in 5% glutaraldehyde in 0.1M phosphate buffer at 4°C and postfixed in 1% OsO_4 in 0.1M phosphate buffer. Samples were dehydrated in ethanol and embedded in an Epon-araldite mixture according to Mollenhauer (1964). Sections were cut with a diamond knife on an LKB Microtome III and double-stained with uranyl acetate and lead citrate. These samples were examined in a Phillips EM301 microscope.

[1]Sorghum Beer Unit, Council for Scientific and Industrial Research, Pretoria 0001, South Africa.
[2]National Physical Research Laboratory, Council for Scientific and Industrial Research, Pretoria.
[3]National Food Research Institute, Council for Scientific and Industrial Research, Pretoria.

0009-0352/83/01002705/$03.00/0

Samples for SEM were fractured with a razor blade. After fracturing, the samples were either freeze-dried or critical-point-dried. The critical-point-dried samples were fixed in 5% gluteraldehyde in 0.1*M* phosphate buffer, dehydrated through a graded ethanol series, and critical-point-dried from amyl acetate. The critical-point-dried samples were coated with gold and used for structure studies, whereas the freeze-dried samples were coated with carbon and used for energy-dispersive X-ray (EDX) analysis. The SEM samples were examined in a JEOL JSM-U3 microscope, and EDX spectra were recorded on a model 711 analyzer supplied by EDAX International Inc.

The polished sections were prepared as follows. After removal of the roots and shoots, the germinated grain was embedded in resin (transparent mounting resin 3, Struers Scientific Instruments, Denmark). The sections were prepared by grinding the embedded grain on emery paper to reveal the desired detail. The sections were finally polished with diamond paste in ethanol.

RESULTS AND DISCUSSION

The grain was germinated for 12 days. This produced a seedling with long roots and shoots, an almost completely empty starchy endosperm, and exhausted or empty aleurone cells. In addition, germination on moist filter paper, rather than conventional malting, allowed us to follow many germination parameters to completion, and the extensive modification made these patterns easier to study. Little mention will be made of absolute germination periods, as different conditions would change the rate of modification. This article is concerned mainly with modification patterns.

Endosperm Modification

Slight modification was visible after the 6-hr steep period. SEM examination revealed that, in the floury endosperm adjacent to the scutellar epithelium, the protein matrix adhering to the starch granules was being modified. It had a light, fluffy appearance instead of the solid, discrete appearance found in the ungerminated grain. After one day of germination, the protein had completely disappeared from this area, and the starch granules were beginning to show pitting.

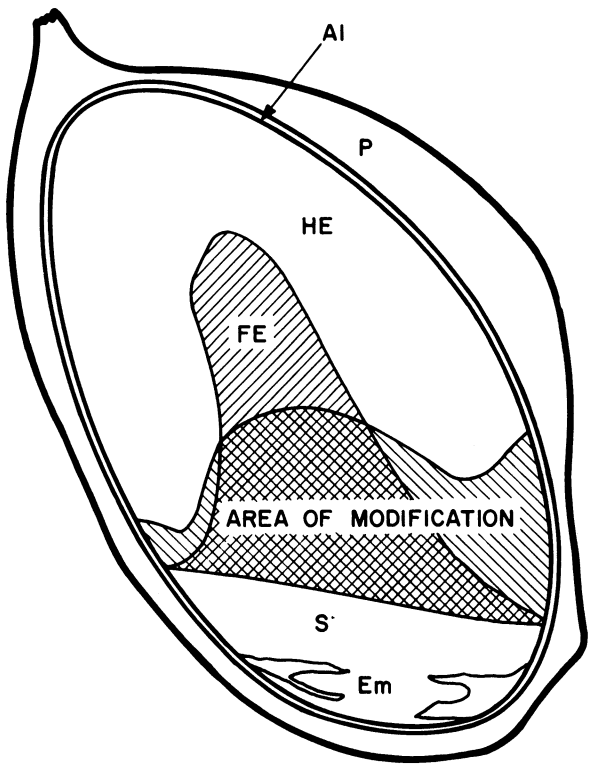

Fig. 1. Pattern of sorghum grain starchy endosperm modification expressed as area of starch granule pitting. Al = aleurone layer, P = pericarp, HE = horny endosperm, S = scutellum, Em = embryo, FE = floury endosperm.

Figure 1 shows the pattern of modification as the area of starch granule modification expanded through the endosperm. Protein matrix degradation appears to follow the same pattern as did attack on starch. First, modification was observed at the endosperm scutellar epithelium interface from where it progressed into the floury endosperm. Simultaneously, the peripheral endosperm adjoining the aleurone layer was slightly modified.

The enzymes responsible for this modification appear to have originated in the scutellum rather than in the aleurone layer. The area of modification did not expand rapidly near the aleurone layer. Rather, it moved uniformly away from the scutellum. This is in agreement with the work of Aisien (1980), who suggested that α-amylase production is localized in the scutellum. Aisien used inhibitors of protein synthesis and showed that α-amylase is synthesized de novo. Koehler (1981) found that more than 90% of the amylase activity occurred in the embryo in germinating sorghum. Cycloheximide and 6-methylpurine inhibited this enzyme increase, which suggested that the enzyme was being synthesized de novo.

The starch granules at the front of the modification area (distal to the scutellum) were slightly pitted. Moving back from the front, the extent of pitting increased until, at the endosperm scutellar epithelium interface, the starch granules were extensively modified. Thus, the microscopic evidence is consistent with the idea that degradative enzymes diffuse out from their origin in the scutellum. This supports the work of Okamoto et al (1980), who found that the scutellar epithelium was the formation site of hydrolases in barley, wheat, rye, oats, and maize.

Aleurone Modification

The aleurone layer, which is also part of the endosperm, was modified during germination. The aleurone layer in sorghum is a single cell thick, whereas in barley it is three to four cells thick. This is not the only difference in the aleurone cells of the two cereals. In germinating barley, the aleurone cells produce α-amylase, and this production can be stimulated by gibberellic acid (Clutterbuck and Briggs 1973). In sorghum, the aleurone cells failed to respond to gibberellic acid (Aisien 1980, Daiber and Novellie 1968. Expanding this hormone study, Aisien (1980) used TLC in an attempt to identify gibberellic acid in germinating sorghum grain. The two hormones GA_1 and GA_3 were absent from sorghum but present in barley. IAA and kinetin were not involved in amylase production in germinating sorghum. Aisien (1980) suggested that the lack of involvement of these hormones in amylase production supports the idea that amylase synthesis does not occur in the aleurone cells. If the aleurone cells in sorghum are not involved in amylase production, then what is their function?

The aleurone layers of both sorghum and barley are rich in minerals. During barley germination, the level of phosphate in the aleurone decreased while the embryo appeared to accumulate phosphate (Clutterbuck and Briggs 1974). Figure 2 shows the mineral content of the sorghum aleurone cells before and after 12 days of germination, as determined by EDX analysis. Although phosphorous was still present after 12 days of germination, the levels of sulfur and potassium were greatly reduced. After 12 days of germination, the aleurone cells were extensively modified. Most of the cell contents were lost, and the cell wall had become modified (Fig. 3). Figure 3A shows how the aleurone cell contents were reduced during the malting period. In the unmalted grain, the contents of aleurone cells were complete, and the cell walls appeared entire (Fig. 3B). Cell wall modification began in the aleurone cell walls adjoining the peripheral starchy endosperm. Figure 3A shows the empty endosperm cells along with their cell walls, which are still intact.

Figure 4 shows a typical cell wall between two aleurone cells in a germinated kernel. Similar plasmodesmata were found in all cell walls joining aleurone cells in both ungerminated and germinated grain. This would facilitate transport of substances between aleurone cells and allow the entire aleurone layer to function as a unit. No plasmodesmata could be found linking the aleurone to the

starchy endosperm. Presumably, the reserves of the aleurone cells migrate to the growing embryo during germination. This migration could take two possible routes. The mobilized reserves could move into the starchy endosperm through the aleurone cell wall, which could suffer a change in permeability or become degraded. Another alternative is that the reserves move from one aleurone cell to the next through the connecting plasmodesmata.

Starch Modification

A typical endosperm cell near the scutellar epithelium in sorghum malted for four days is shown in Fig. 5. The matrix protein was digested, and the starch granules were extensively pitted. Even though pitted, the starch granules retained their shape until germination was well advanced; only then did the granules collapse to reveal their hollow interiors. Figure 6 shows a modified starch granule with one end polished away to reveal the hollow interior with the clearly defined interior concentric sphere of the granule. The deposition and chemistry of starch granules and their concentric sphere structure were previously reported (Buttrose 1960).

Sorghum starch granules were similar to the large starch granules in barley in that they were attached by pitting rather than by the surface erosion characteristic of small starch granules of barley (MacGregor 1980). In the sorghum used in this study, smaller starch granules were found in the pericarp, but they remained intact even after 12 days of germination.

Barley and sorghum are similar in that the protein matrix began to disappear during steeping. In sorghum, the cell walls did not disappear as they did in barley (MacGregor 1980). Figure 7 shows the endosperm cell walls and their structure after the cells became empty. This is in contrast to barley, in which the cell walls disappeared during the early stages of malting (Morrall and Briggs 1978).

In the SEM study, critical-point-drying preserved the cell wall structure much better than did freeze-drying. On the other hand, the freeze-dried samples contained considerably more starch in the endosperm than the critical-point-dried samples. It was difficult to observe what effect the different drying regimes had on cell contents, except that critical-point-dried aleurone cells appeared different from the freeze-dried cells. More work is being done on the chemical composition of the endosperm cell walls to determine what changes occur during the malting period. During germination the starch granules became partially digested, and the intergranular protein matrix was modified, allowing the starch granules to become free. This loose starch was probably washed away in the

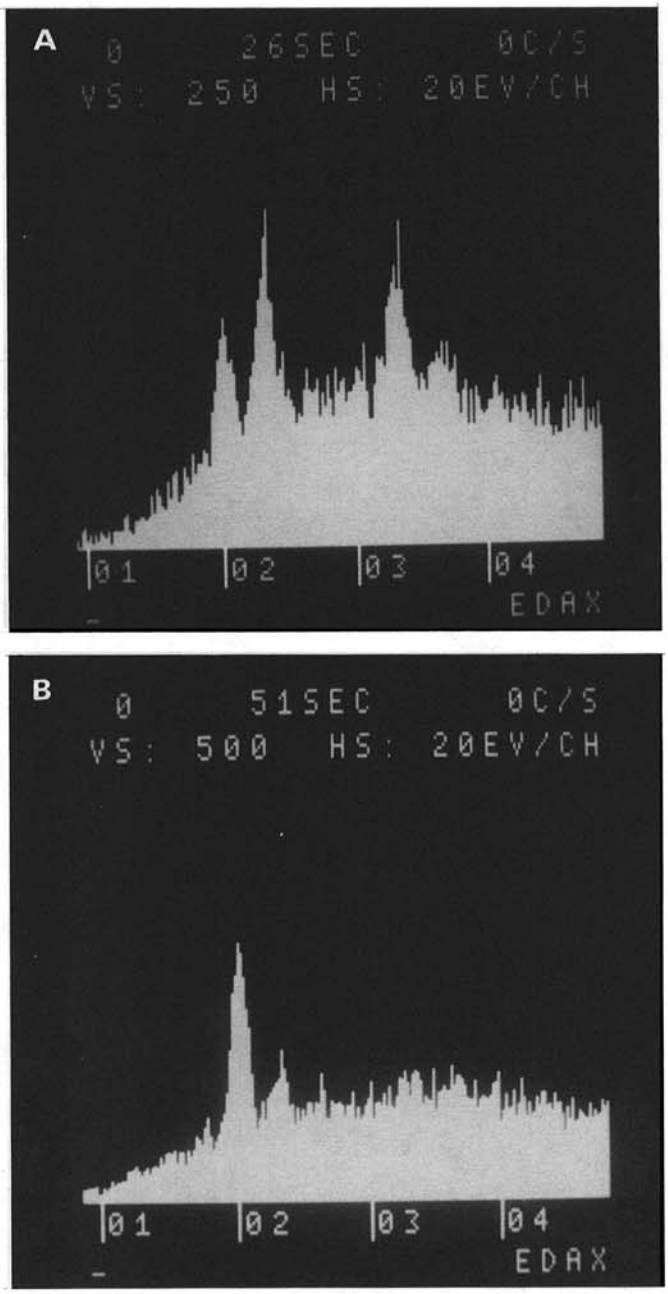

Fig. 2. EDX analysis of aleurone cells of C-coated sorghum grain. **A,** 0-day germinated grain showing the presence of P, S, and K. (P_K, 2.013 keV; S_K, 2,307 keV; $K_{K\alpha}$, 3.312 keV; $K_{K\beta}$, 3.589 keV). **B,** 12-day germinated grain showing the presence of P and S.

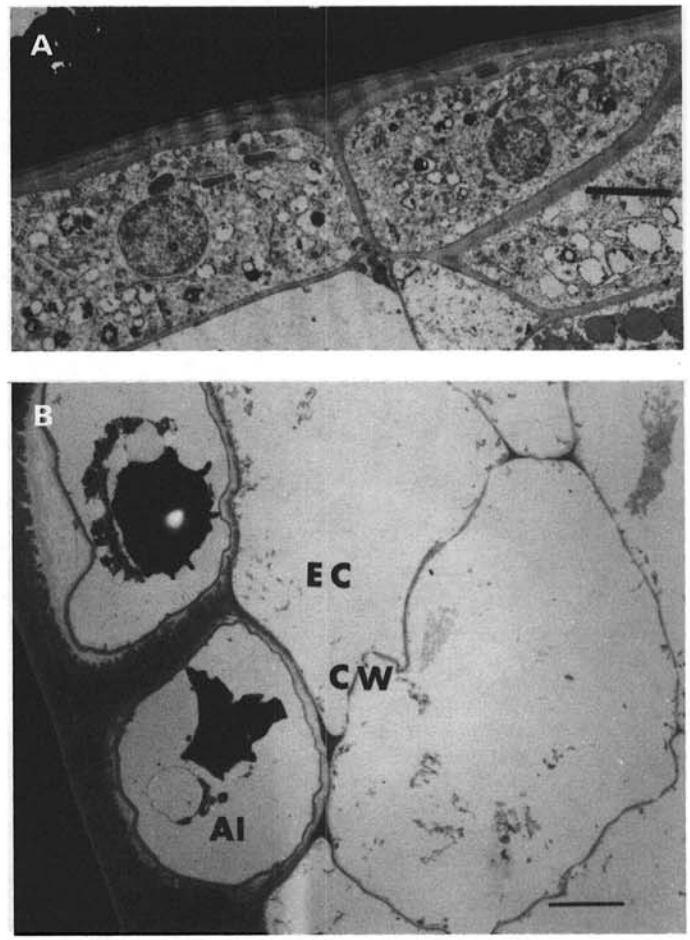

Fig. 3. A, Aleurone cells in preharvest (hard-dough stage) sorghum grain showing cell contents and complete cell walls. Bar = 5 μm. **B,** Aleurone cells and adjacent peripheral endosperm after 12 days of germination. Both types of cells have lost their cell contents and the aleurone cell walls have been modified. Al = aleurone cell, EC = endosperm cells, CW = cell wall. Bar = 5 μm.

critical-point-drying procedures, causing the cells to be empty (Fig. 7).

Protein Modification

By the term "protein bodies," we mean those bodies found in the starchy endosperm and composed mainly of protein. Aleurone grains are those organelles found in the aleurone cells and composed of a protein matrix in which are embedded crystalloid proteins and phytate globoids (Pernollet 1978).

Protein bodies in sorghum can be found throughout the horny endosperm, with the greatest concentration in the peripheral areas (Seckinger and Wolf 1973). In these areas, a large amount of matrix was extensively degraded during germination before the protein bodies were usually degraded (Fig. 8). The amount of matrix protein appeared to be reduced, and that which remained had a reticulate appearance. The protein bodies lost their closely packed arrangement. Simultaneously with protein digestion, the adjacent starch granules became degraded (Fig. 9). This pattern of degradation can be found throughout the regions of modification. A similar pattern of protein breakdown was observed in a biochemical study involving sorghum. Wu and Wall (1980) found that the prolamins, which make up the bulk of the protein bodies, decreased during the germination period.

Protein body digestion may have two patterns: internal digestion (from within); or external digestion, where the peripheral areas are degraded first (Ashton 1976, Novellie et al).[4] In the present study,

[4]L. Novellie, K. C. Butler, and T. C. Robberts. 1978. The biochemistry of germinating sorghum. Presented at the Sixth International Cereal and Bread Congress, Winnipeg, Canada.

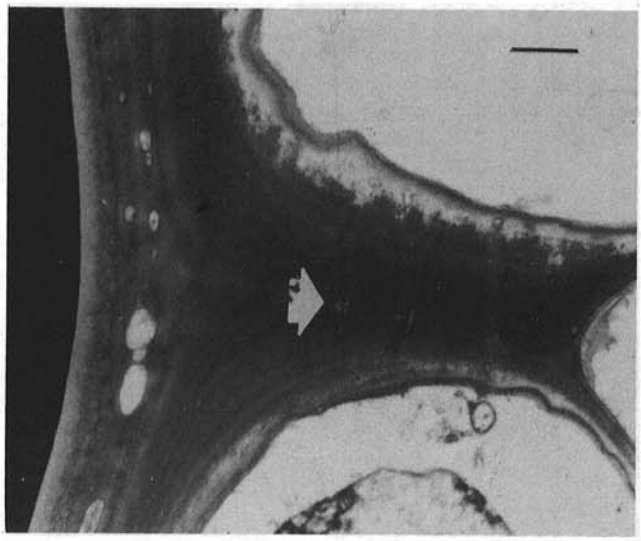

Fig. 4. Cell wall between aleurone cells showing plasmodesmata (arrow) and cell wall modification after 12 days of germination. Bar = 1 μm.

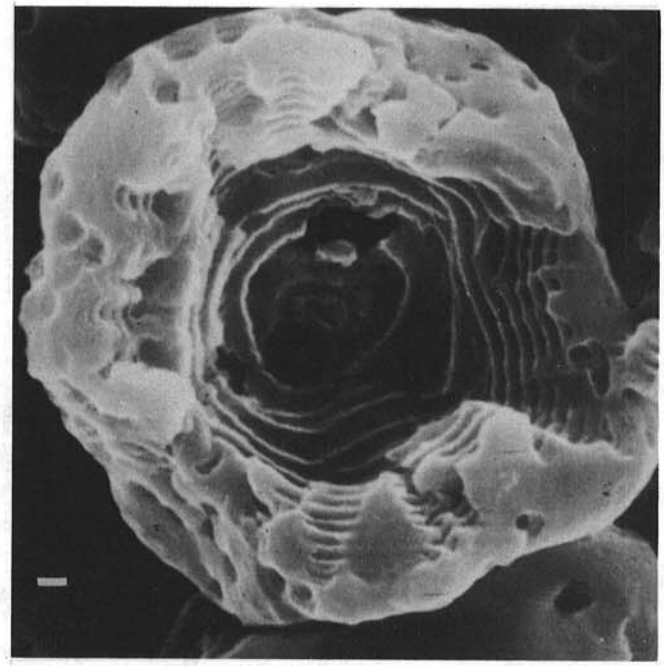

Fig. 6. Polished section of partially digested starch granule (seven-day malt). Bar = 1 μm.

Fig. 5. Floury endosperm adjacent to scutellar epithelium after four days of germination showing pitted starch granules and intact cell walls. S = starch granule, CW = cell wall. Bar = 10 μm.

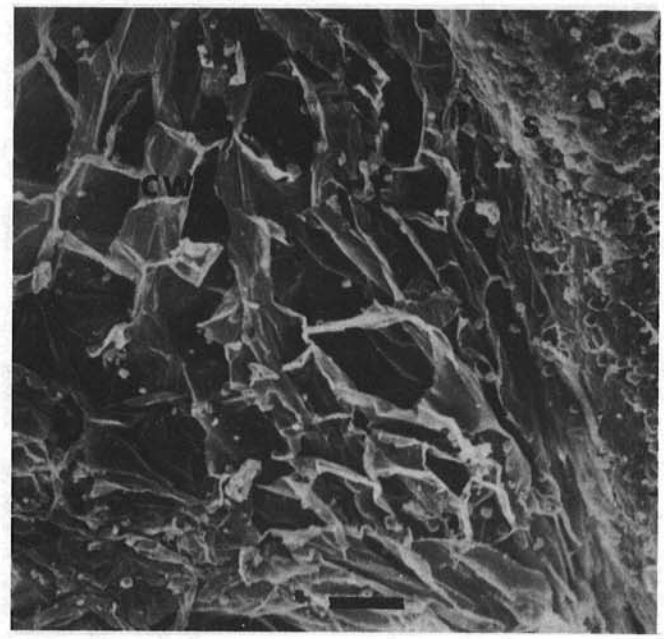

Fig. 7. Floury endosperm adjacent to scutellar epithelium showing empty cells with intact cell walls (ten-day malt). CW = cell wall, S = scutellum. Bar = 100 μm.

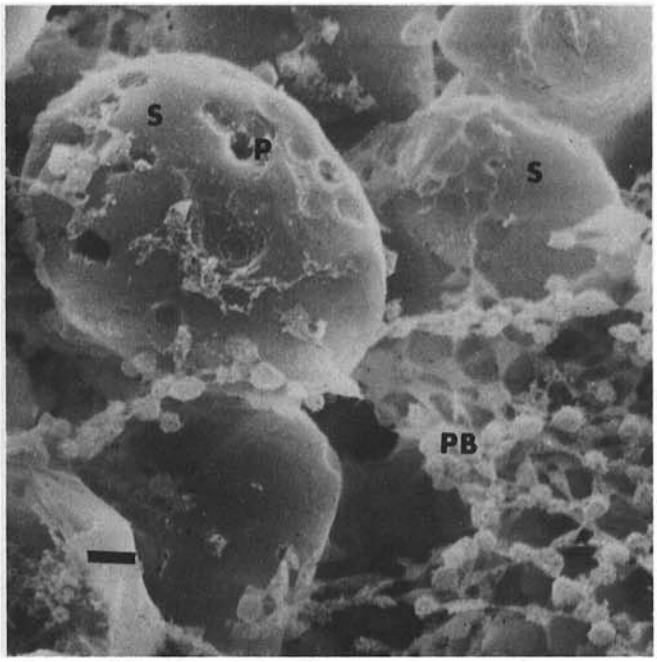

Fig. 8. Peripheral endosperm showing pitted starch granules and partially modified protein bodies with residual strands of protein matrix (four-day malt). S = starch granule, P = pitting, PB = protein bodies. Bar = 2 μm.

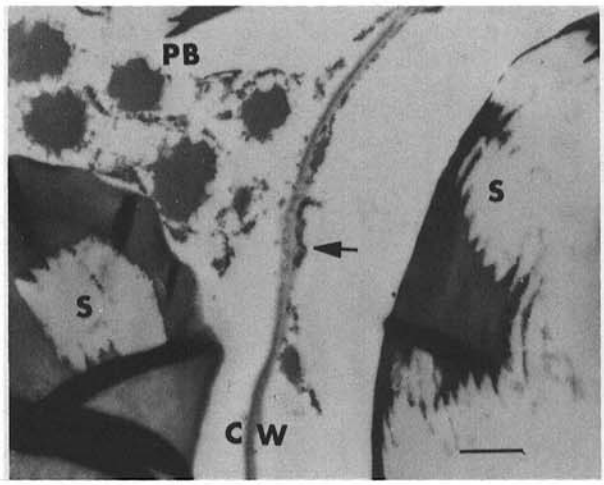

Fig. 9. Transmission electron photomicrograph of modified starch granules and protein bodies in horny endosperm (four-day malt). Arrow = intact cell wall with plasmodesmata. S = starch granule, PB = protein body, CW = cell wall. Bar = 1 μm.

the majority of protein bodies appeared to be broken down from the exterior. However, in some sections, the protein bodies appeared to have internal areas of digestion. These areas may represent external digestion areas that occur at different planes to the section. This phenomenon will have to be examined further to determine the origin of the proteolytic enzymes, ie, do they come from the scutellum or aleurone, or are they zymogens that are activated in situ?

SUMMARY

In germinating sorghum grains, the modification patterns of the endosperm appeared to be controlled by the scutellum. The aleurone cells did not appear to contribute to the modification of the starchy endosperm, but they certainly became extensively modified, and their mineral content was reduced. The endosperm cell walls remained intact, and the only visible modification of cell walls in situ occurred around the aleurone cells.

LITERATURE CITED

AISIEN, A. O. 1980. Ultrastructure of sorghum and millet grains in relation to germination. Ph.D. thesis. Heriot-Watt University, Edinburgh, Scotland.

ASHTON, F. M. 1976. Mobilization of storage proteins of seeds. Annu. Rev. Plant Physiol. 27:95.

BOTES, D. P., JOUBERT, F. J., and NOVELLIE, L. 1967a. Kaffircorn malting and brewing studies. XVII. Purification and properties of sorghum malt α-amylase. J. Sci. Food Agric. 18:490.

BOTES, D. P., JOUBERT, F. J., and NOVELLIE, L. 1967b. Kaffircorn malting and brewing studies. XVIII. Purification and properties of sorghum malt β-amylase. J. Sci. Food Agric. 18:415.

BUTTROSE, M. S. 1960. Submicroscopic development and structure of starch granules in cereal endosperms. J. Ultrastruct. Res. 4:231.

CLUTTERBUCK, V. J., and BRIGGS, D. E. 1973. Enzyme formation and release by isolated barley aleurone layers. Phytochemistry 12:537.

CLUTTERBUCK, V. J., and BRIGGS, D. E. 1974. Phosphate mobilization in grains of *Hordeum distichon*. Phytochemistry 13:45.

DAIBER, K. H., MALHERBE, L., and NOVELLIE, L. 1973. Sorghum malting and brewing studies. Part 22. The modification of sorghum malt. Brauwissenschaft 26:220, 248.

DAIBER, K. H., and NOVELLIE, L. 1968. Kaffircorn malting and brewing studies. XIX. Gibberellic acid and amylase formation in kaffircorn. J. Sci. Food Agric. 19:87.

HOSENEY, R. C., DAVIS, A. B., and HARBERS, L. H. 1974. Pericarp and endosperm structure of sorghum grain shown by scanning electron microscopy. Cereal Chem. 51:552.

JAYATISSA, P. M., PATHIRANA, R. A., and SIVAYOGASUNDERAN, K. 1980. Malting quality of Sri Lankan varieties of sorghum. J. Inst. Brew. 86:18.

KOEHLER, D. E. 1981. Hydrolytic enzyme production during sorghum germination. (Abstr.) Plant Physiol. 67 (suppl.):218.

MacGREGOR, A. W. 1980. Action of malt α-amylase on barley starch granules. Master Brew. Assoc. Am. Tech. Q. 17:215.

MOLLENHAUER, H. H. 1964. Plastic embedding mixture for use in electron microscopy. Stain Technol. 39:111.

MORRALL, P., and BRIGGS, D. E. 1978. Changes in cell wall polysaccharides of germinating barley grains. Phytochemistry 17:1495.

NOVELLIE, L. 1960a. Kaffircorn malting and brewing studies. IV. The extraction and nature of the insoluble amylases of kaffircorn malts. J. Sci. Food Agric. 11:408.

NOVELLIE, L. 1960b. Kaffircorn malting and brewing studies. V. Occurrence of β-amylase in kaffircorn malts. J. Sci. Food Agric. 11:457.

NOVELLIE, L. 1968. Kaffir beer brewing: Ancient art and modern industry. Wallerstein Lab. Commun. 31:17.

OKAFOR, N., and ANICKE, G. N. 1980. Brewing a lager beer from Nigerian sorghum. Brew. Dist. Int. 10:32.

OKAMOTO, K., KITANO, H., and AKAZAWA, T. 1980. Biosynthesis and excretion of hydrolysates in germinating cereal seeds. Plant Cell Physiol. 21:201.

PALMER, G. H. 1980. The morphology and physiology of malting barleys. Page 301 in: Cereals for Food and Beverages. G. E. Inglett and L. Munck, eds. Academic Press, New York.

PALMER, G. H., and BATHGATE, G. N. 1976. Malting and brewing. Page 237 in: Advances in Cereal Science and Technology. Vol. I. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

PERNOLLET, J. C. 1978. Protein bodies of seeds: Ultrastructure, biochemistry, biosynthesis, and degradation. Phytochemistry 17:1473.

SECKINGER, H. L., and WOLF, M. J. 1973. Sorghum protein ultrastructure as it relates to composition. Cereal Chem. 50:455.

SULLINS, R. D., and ROONEY, L. W. 1975. Light and scanning electron microscopic studies of waxy and nonwaxy endosperm sorghum varieties. Cereal Chem. 52:361.

WU, Y. V., and WALL, J. S. 1980. Lysine content of protein increased by germination of normal and high-lysine sorghums. J. Agric. Food Chem. 28:455.

[Received March 22, 1982. Accepted August 20, 1982]