# Cereal Chemistry

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## The Microscopic Structure of Popped Cereals[1]

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**ABSTRACT**

The endosperms of grains expanded by popping show different degrees of starch granule gelatinization. In barley and wheat, which do not expand greatly, some starch granules undergo complete gelatinization without appreciable granule expansion; other gelatinized granules appear to have expanded and fused. Localized cell-wall rupturing occurs when the kernels split open; a few intracellular voids or enlarged bubbles occur in the gelatinized starch as a result of popping explosion. Ungelatinized and partly gelatinized starch granules predominate immediately beneath the aleurone and near the scutellum. Localized cell-wall rupturing also occurs in expanded endosperms of popped grain sorghum or milo, popcorn, and dent corn, but the spongy, expanded endosperms consist of intact cells within which the gelatinized starch forms a characteristic "soap-bubble" structure. Gelatinized granules expand directly to form the individual bubbles. Expansion is much less pronounced in dent corn than in milo and popcorn, in which more cell rupturing occurs to form voids. The soap-bubble structure also is less extensively developed in all poorly popped kernels. Some unaltered and partly gelatinized starch is present immediately beneath the aleurone and near the scutellum, even in fully popped kernels. Differences in distribution of horny and floury endosperm, and differences in their protein content influence the capacity to expand when different cereals are popped. There appear also to be some differences between starch granules. More fundamental information on starch composition in different cereals is needed for a more complete understanding of interrelationship between protein, starch, and structure in relation to popping expansion.

Almost all recent cattle-feeding studies show that use of processed grain in high-concentrate rations improves feed efficiency. A variety of processing methods have been proposed (1,2,3,4,5), including hot air expansion or popping (6). During investigations on the use of popped barley and wheat in cattle feeds, we found that very little has been published concerning the microscopic structure of popped grains and that there are no descriptions of the conditions of starch in popped barley and wheat. Carr and Ripley (7) have illustrated popped popcorn in microscopic section, but their brief descriptions provide little histological information and include no comparisons with other popped cereals. Weatherwax (8) described kernel structure as related to popping and considered that properties of the protein in the horny endosperm influenced popping expansion. Herzka (9) has illustrated the structure and

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starch distribution of maize as related to popping. However, information as to the relation of ease of popping to histological characteristics and starch properties in different grains is very meager.

This paper describes some of the histological changes which result in several cereals when they are popped, and also provides some comparisons as to completeness of popping in relation to observable differences in their starches.

## MATERIALS AND METHODS

Samples of barley, wheat, dent corn, grain sorghum or milo, and popcorn were popped at this Laboratory by exposure to temperatures of 475°F. in a stream of dry air in a Surface Combustion pilot toaster. Optimum conditions for maximum popping varied for each kind of grain because of variations in moisture contents and other compositional factors, so that a wide variation in degree of expansion resulted.

Samples of the raw grains as well as those representing various degrees of popping expansion were prepared for histological study. A modification of the method of Larkin *et al.* (10) was used for raw grains, employing xylene rather than chloroform as the paraffin solvent. The grains were killed and fixed for 24 hr. in CRAF solution, then soaked overnight in water. Some were then cut in half longitudinally, median to the germ, and others were cut transversely just above the germ before treatment with CRAF. All samples were prestained as an aid to orientation when embedded. This was accomplished with safranin in the 75% xylene-25% alcohol step of the replacement schedule.

Softening of embedded samples for sectioning also was done by a modification of the method of Larkin *et al.* (10). After paraffin had been trimmed away to expose the cut surface of each sample, the blocks of embedded material were submerged 24 to 72 hr. in a solution of 1 part glycerine, 1 part glacial acetic acid, and 10 parts 75% ethyl alcohol prior to sectioning at 15 and 20 $\mu$. Other samples, particularly those poorly popped, were prepared by histological procedures variously modified from the method of Larkin *et al.* (10), and embedded in paraffin, m.p. 52°C., for sectioning on a rotary microtome. Some were processed in the same manner as were soaked raw grains. Others were vacuum-infiltrated with a 10% aqueous solution of commercial formaldehyde for about 1 hr. prior to the alcohol-xylene schedule. However, use of aqueous solutions caused various degrees of swelling of gelled starch in the fully expanded kernels; this sometimes resulted in pronounced artifacts in sections cut after paraffin-embedment.

To minimize these artifacts, some popped samples were partially vacuum-infiltrated with 60% ethyl alcohol containing about 5% of commercial formalin. They were then passed through three or four changes of absolute alcohol, 2 to 3 hr. each, before stepwise replacement of the alcohol with xylene. After three changes of xylene, paraffin was infiltrated with floating cubes of the aerated wax to ensure uniformly gradual infiltration until saturation was reached at 40°C. Four changes of pure paraffin (1 day each) were then made before embedding. Although even prolonged infiltration did not

remove sufficient trapped air to prevent the samples from floating in melted paraffin, paraffin impregnation was sufficient to permit reasonably good sections to be cut without trimming and soaking to soften the tissues.

Sections about 100 $\mu$ thick could be readily cut by hand with a razor blade from the fully popped kernels. Sections uniformly 80 $\mu$ thick were obtained with a sliding microtome by clamping samples between strips of pith. After the first cuts were made, the cut surfaces were brushed with a 2% parlodion (cellulose nitrate) solution which was allowed to dry before each section was cut.

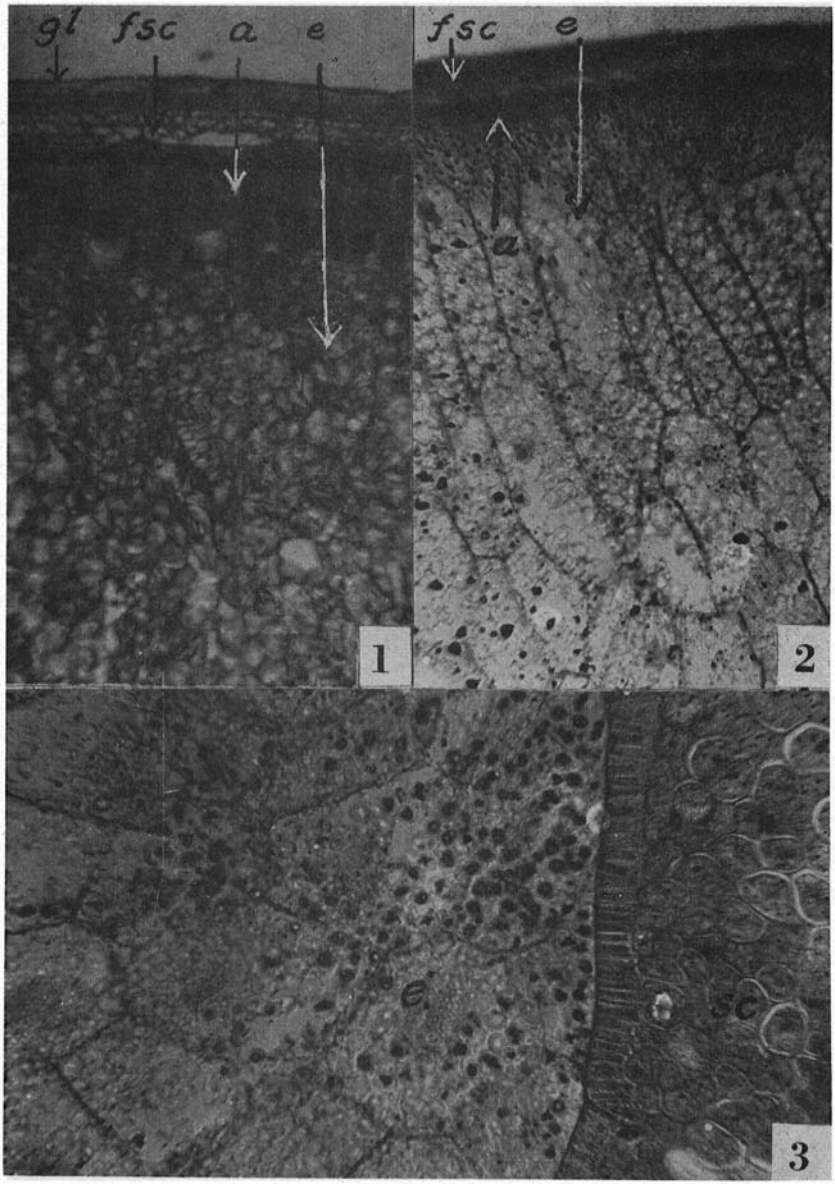
Such sections could be readily vacuum-infiltrated with alcohol, xylene, or water to remove entrapped air. Some were mounted unstained in Hyrax, which has a refractive index of about 1.65, to enhance boundary detail in the starch. Those, mounted in media of lower refractive indices, were first stained in a mixture of safranin and thionin in a 40% alcohol and 60% xylene mixture. Before mounting, the sections were passed through two or three changes of xylene. Sections vacuum-infiltrated with water were mounted in a glycerine-jelly preparation containing about 0.2% methylene blue to stain cellulosic walls.

Sections of all paraffin-embedded samples were floated onto microslides precoated with a thin film of Haupt's gelatin adhesive, over which several drops of 3% commercial formaldehyde solution were spread. The sections were then expanded and dried on a warming plate at 40°C. Although this resulted in some irregular expansion of sections of popped grains, the resulting folds and other irregularities in the sections were not too extensive, and areas free from such artifacts could be readily selected for observation. Staining of raw grain sections was done with an adaptable schedule (11), principally with Delafield's hematoxylin, safranin, and fast green. Many of the sections of embedded popped grains were also mounted unstained in Hyrax. Those mounted in resins of lower refractive indices were first stained with safranin, thionin, and hematoxylin in the alcohol-xylene mixture after removal of paraffin with xylene. This staining procedure avoided distortion due to swelling of gelatinized starch in aqueous solutions of dyes, as are more conventionally used. Iodine and other stains also were used on thick sections cut without embedment. Microscopic examinations were made with both ordinary illumination and with the polarizing microscope.

## OBSERVATIONS

### Raw Grain Anatomy

The origins, histological structure, and composition of tissues comprising cereal grains are well known (12,13,14–16,17–21), and starch granule formation in several cereals has been studied in detail (22,23). In addition, detailed descriptions of mature grain histology have been published for dent corn and wheat (19,20,24,25). All cereal grains have certain common morphological features; they differ mainly in composition, size, shape, and extent of development of component parts. For example, barley, as ordinarily used for a feed, retains the flower glume and palea appressed to the kernel. Also, barley aleurone has two to four cell layers. These features are shown




Figs. 1, 2, and 3. Fig. 1: 20-$\mu$ section of raw barley (times 120) showing flower glume (gl), fruit coat or pericarp and seed coat (fsc), multilayered aleurone (a) and endosperm (e). Section mounted in Hyrax to enhance starch detail which obscures thin cell walls of endosperm.

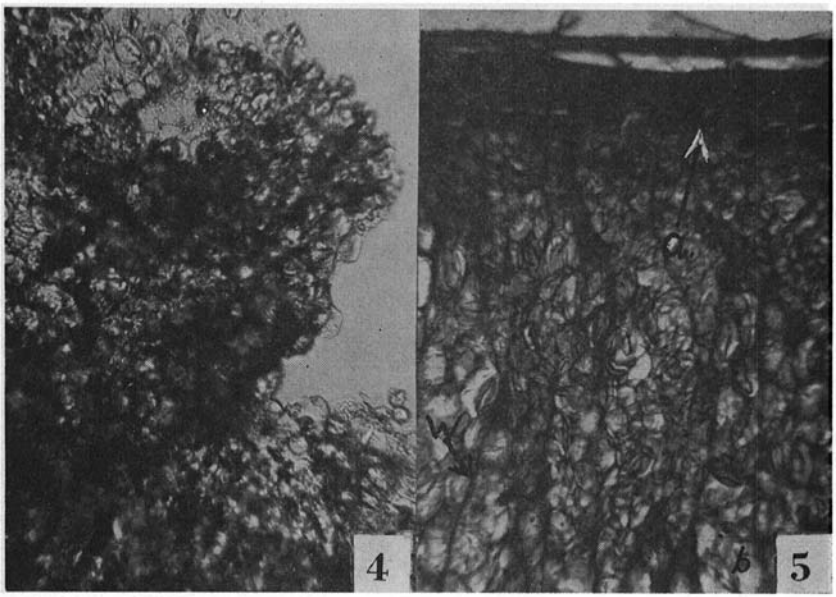
Figs. 2 and 3 are 20-$\mu$ sections of raw milo (times 120) stained and mounted in Custo-mount. Fig. 2: Section of lateral side of kernel, showing pericarp and seed coat tissues (fsc), small-celled, single-layered aleurone (a), and endosperm (e). Black dots are air bubbles trapped in the hilum cracks of starch granules. Fig. 3: Section internal to grain at border of scutellum (sc) and endosperm (e).

in Fig. 1 by a section mounted in Hyrax, which has obscured the cell walls of the endosperm. The starch granules are round to oval, as is also characteristic of wheat starch.

Figures 2 and 3 are of milo, a naked-kernel cereal grain. The single-layered aleurone consists of much smaller cells than in most common grains. The small, tightly packed endosperm starch granules are polygonal to slightly rounded, as is also characteristic of dent corn and popcorn starches. In general, the endosperm cells of all common grains are polygonal and vary from nearly isodiametric to distinctly elongated shapes.

**Popped Barley and Wheat**

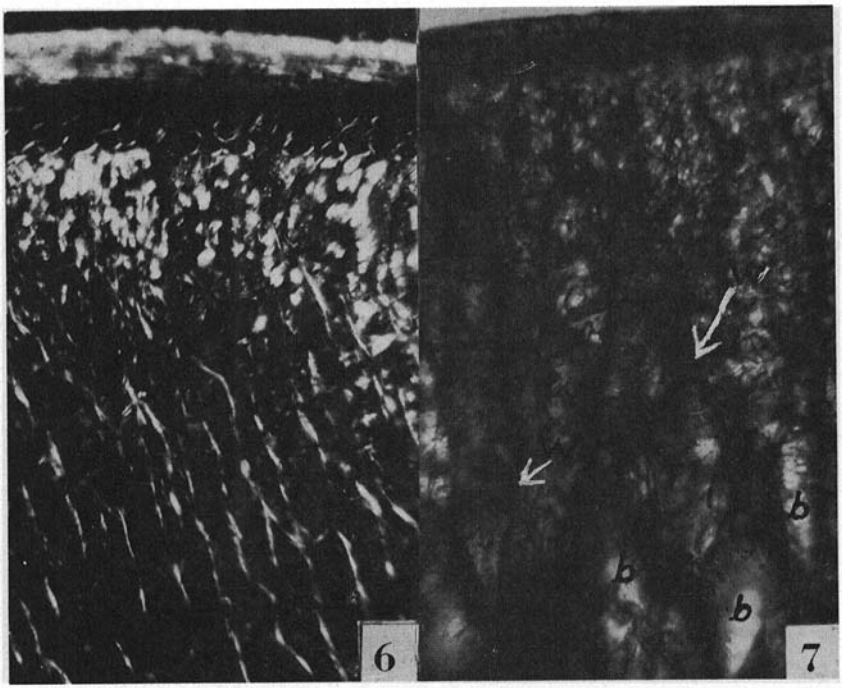
Only slight popping expansion occurred with the samples of barley and wheat used here, and this varied from only slightly swollen and cracked kernels to those opened and expanded to about twice their original size. When popped, neither barley nor wheat exhibited anything comparable to the reticulum of gelatinized starch formed by individual starch granules of the corn varieties, as described later. Many of the starch granules in the edges of expanded barley and wheat endosperm appeared to be gelatinized but not greatly swollen (Fig. 4). A few contained very minute trapped air bubbles. Most of entrapped air, however, appeared to be spread through the cellular contents as, perhaps, between gelatinized starch granules that had more or less fused before pronounced gas expansion had occurred. Thus, bubbles or small intracellular voids of various sizes were formed (Figs. 5, 7, 8, 9).



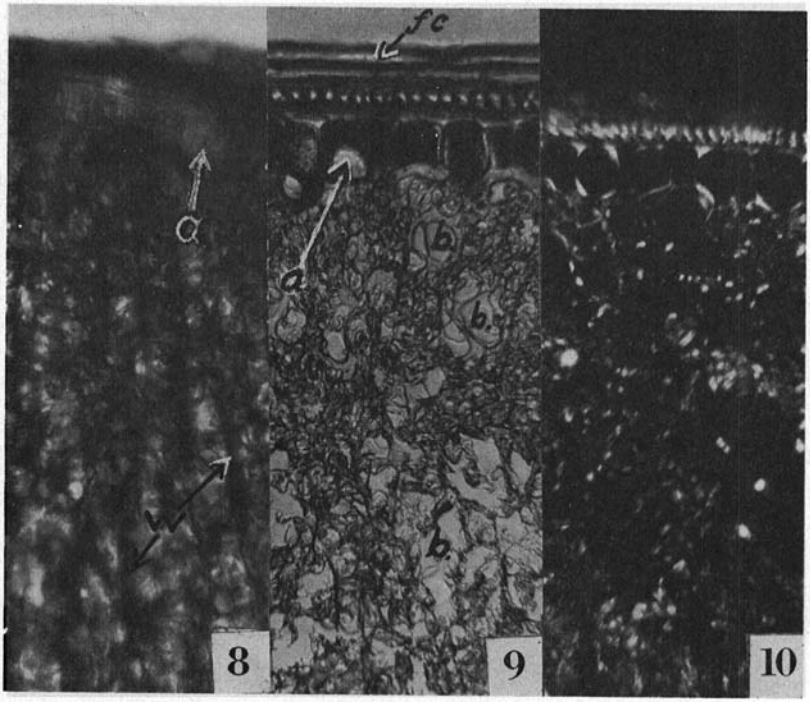

Figs. 4 and 5. Sections of popped barley (times 120). Fig. 4: 80-$\mu$ section at edge of expanded endosperm, showing ungelatinized, partly and fully gelatinized starch; mounted in Hyrax. Fig. 5: 20-$\mu$ section from embedded sample, showing structural condition underlying multilayered aleurone; stained and mounted in Customount. Most of the starch granules near the aleurone are ungelatinized.

Careful examination of thin serial sections revealed that at least some of these small intracellular voids appeared to interconnect.

Cellular structure was well preserved in the endosperms of both popped barley and popped wheat, particularly underlying the aleurone (compare Figs. 5, 6, and 7 with 8 to 10). The irregular intracellular voids formed by trapped air were evident in both thinner sections and thicker ones that were vacuum-infiltrated (Figs. 7 and 9). Unaltered and partly gelatinized starch granules appeared to be scattered in popped wheat but were more localized under the aleurone of popped barley (Figs. 6 and 10). Both unaltered and gelatinized starch granules often occurred within the same cells of wheat endosperm (Fig. 10), and were occasionally found within individual cells of other cereals studied. Many of the less swollen starch granules exhibited some birefringence when examined with the polarizing microscope, particularly at margins of the starch granules, but no typical dark crosses of unaltered starch granules. It would seem that removal of water, either by flash evaporation during popping or by alcoholic dehydration during histologic preparation, has resulted in some molecular reassociation in some of the swollen granules.




Figs. 6 and 7. Fig. 6: This section is similar to that shown in Fig. 5, but was photographed with polarizing microscope, showing ungelatinized starch (bright) underlying aleurone. Bright lines beneath the ungelatinized starch are cell walls of endosperm cells in position of brightness between crossed nicols. Fig. 7: 80-$\mu$ section stained and mounted in Hyrax. a, aleurone; b, air spaced in gelatinized starch matrix (note absence of reticulum); w, cell walls.



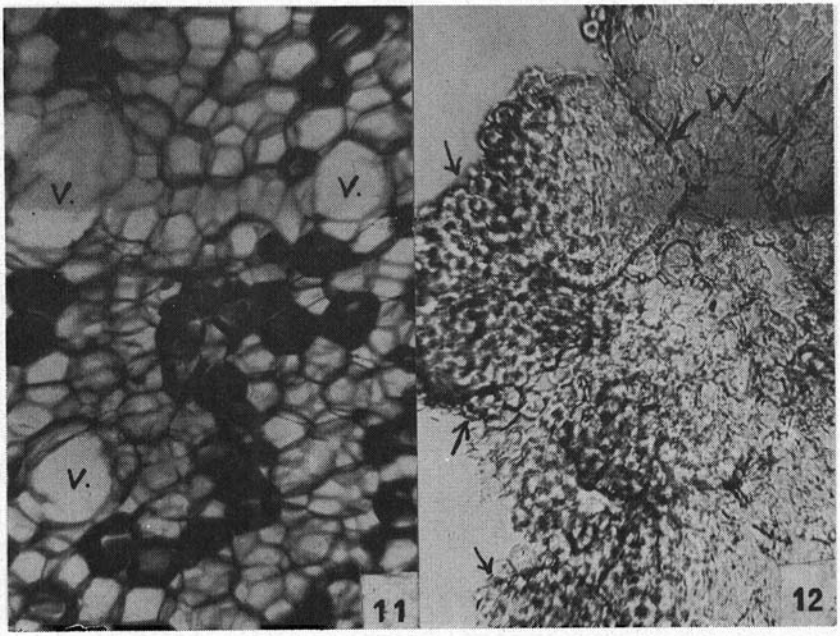

Figs. 8 to 10, sections from popped wheat (times 120). Fig. 8: 80-$\mu$ section stained and mounted. Fig. 9: 20-$\mu$ section from embedded sample, stained and mounted in Hyrax. Fig. 10: same as 9 but photographed with polarizing microscope. All sections cut from lateral parts of popped grains near areas cracked by expansion. a, aleurone; b, air-space bubbles in gelatinized starch matrix, fc, pericarp and seed-coat tissues; w, cell walls of endosperm cells.

MacMasters (26) described return of birefringence in several gelatinized starches, including barley, corn, and wheat, gelatinized by heating at 10° to 15°, and occasionally 30°C. above the temperature at which they would normally lose birefringence. She found that return of birefringence with ethanol treatment was confined to outer portions of the swollen granules, or that area in which birefringence tended to persist until the granules double or triple their original size. It did not occur with waxy corn and glutinous rice starches.

Although the conditions under which MacMasters effected starch gelatinization differ from those of popping, some of the phenomena resulting are remarkably parallel. Similar conditions of partial birefringence were also observed in the endosperm starch granules of poorly popped milo and popcorn in which there also was a greater amount of ungelatinized starch than in well-popped kernels.

#### Popped Milo, Dent Corn, and Popcorn

The structural appearance and gelatinized starch contents of the expanded endosperms of popped milo and popcorn contrast strongly with those of



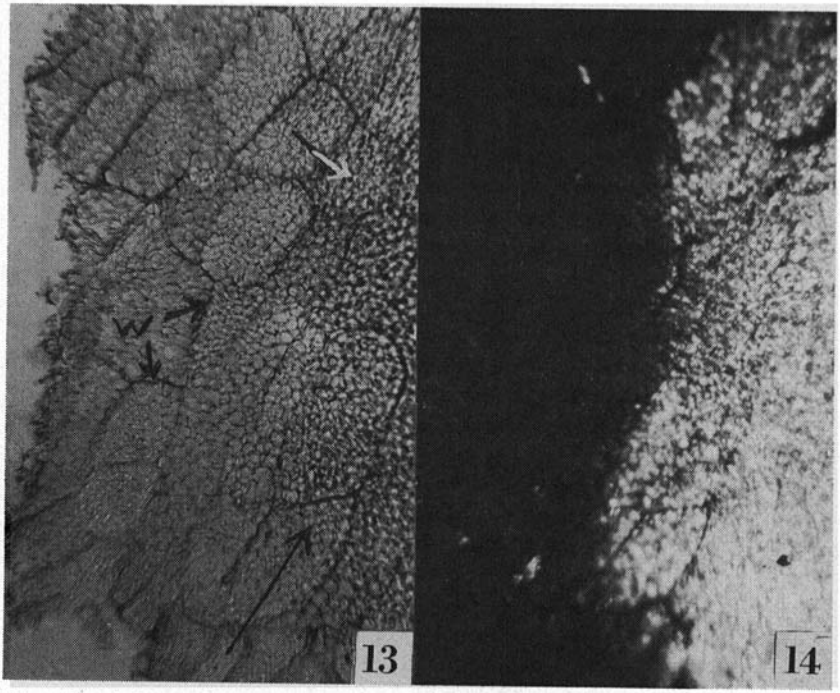

Figs. 11 and 12, sections of expanded endosperms (times 120). Fig. 11: from popped milo, 80 $\mu$ thick and mounted in Hyrax to show soap-bubble reticulum of gelatinized starch. Fig. 12: from popped milo near scutellum, 80 $\mu$ thick, and mounted in glycerin jelly.

wheat and barley. If wheat and barley represent a range from slight to intermediate degrees of popping, then fully popped milo and popcorn represent the extreme degree. In addition, although dent corn did not greatly expand upon popping, its gelatinized starch content was comparable with that of popped milo and popcorn.

Figures 11 to 18 compare the structural conditions of gelatinized starch contents of expanded endosperms of milo, dent corn, and popcorn. In the more expanded portions, such as would represent most of the central parts of the endosperm of the raw grain, the gelatinized starch is reticulate in section view (Figs. 11 to 13 and 15 to 17). Some larger voids due to cell rupturing are present (Figs. 11 and 15) and, under conditions of only partial vacuum infiltration of the sections, some air bubbles remain trapped within the starch gel reticulum.

In thicker sections mounted in Hyrax, the reticulum is comparable to fine, polyhedral soap bubbles confined in an enclosure (Figs. 11 and 15). Each bubble represents an individual starch granule, gelatinized and expanded by heat, then dried and left hollow upon release of internal steam pressure, as shown in Fig. 12. When sections of each tissue were mounted in water the gelatinized starch walls of the bubbles swelled and the bubbles tended to become more spherical, appearing like doughnuts in median optical view (Fig. 16).

Although expansion of the popped dent corn samples studied was only

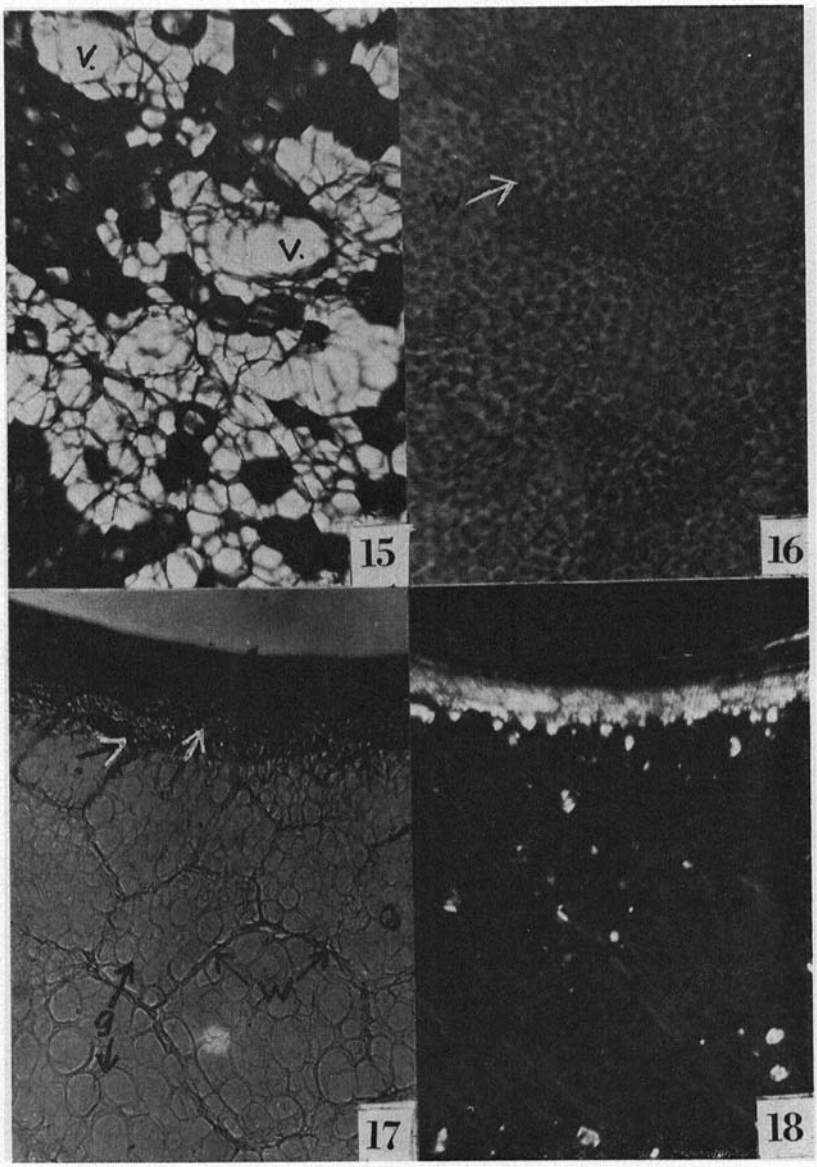



Figs. 13 and 14, sections of expanded endosperms (times 120). Fig. 13: from dent corn section, 20 $\mu$ thick from embedded sample sectioned at edge of crack in expanded endosperm, stained and mounted in Customount. Fig. 14: same as 13 but photographed with polarizing microscope. Arrows, ungelatinized starch; v, voids; w, cell walls.

slight by comparison with milo and popcorn, the starch gel reticulum was clearly evident in thin sections through marginal cracks of the expanded endosperm (Fig. 13). Deeper beneath this margin the starch granules remained ungelatinized and were birefringent in the polarizing microscope (Fig. 14). Large voids, as formed by cell-rupturing in the expanded endosperm, were seldom encountered in popped dent corn.

Cellular structure of the endosperm was remarkably well preserved despite its pronounced expansion, particularly in the more fully popped milo and popcorn (compare Figs. 12, 13, 16, and 17). Although some cell-size difference exists between these three cereal grains, much of the difference illustrated here is relative to area of endosperm sectioned and degree of expansion. Cell walls and endosperm cells remained largely intact, as shown in thick, glycerine jelly-mounted sections as well as in thinner sections of embedded samples (Figs. 13, 16, and 17).

Some cell-rupturing and cell-separation was evident within the more expanded parts of the endosperm. Thicker sections often contained voids (Figs. 11 and 15) and, in thinner sections from embedded samples, such regions often showed even more structural disintegration, owing in part to swelling of the starch gel as the paraffin sections expanded when floated onto the microslide.




Figs. 15 and 16. 80-$\mu$ sections of expanded endosperm of popcorn (times 120). Fig. 15: mounted in Hyrax; v, void. Fig. 16: mounted in glycerin jelly; w, cell walls.

Figs. 17 and 18. 20-$\mu$ sections from embedded sample of popped milo, showing structural condition underlying the pericarp and aleurone; mounted in Customount (times 60). Fig. 17: photographed with ordinary illumination. Arrows, ungelatinized starch; g, gelatinized starch reticulum; w, cell walls. Fig. 18: same as 17 but photographed with polarizing microscope.

These findings are contrary to the interpretation advanced by Weatherwax (8) who stated: "Physical examination shows a profound change in the texture of the endosperm, the cell walls being destroyed, the starch grains exploded, and other characteristics of organic structure obliterated . . ." Obviously, the cell walls are not destroyed and they remain clearly identifiable, except where wall-rupturing contributed to both expansion and formation of voids. Also, the starch granules are not exploded, but rather are gelatinized and dried into a network or three-dimensional reticulum.

Cell walls of the endosperm cells near the aleurone layer remained mainly intact, although some swelling of endosperm cells was often evident. Starch granules in cells subjacent to the aleurone were either unaltered or partly gelatinized (Figs. 17 and 18). Likewise, in deep endosperm areas near the scutellum, the starch granules in milo and popcorn remained ungelatinized and their highly refractive condition often caused cracking or tearing during cutting of sections. Only in those areas where expansion had ruptured the aleurone and outer layers was there any appreciable damage to cellular structure. Exposed surfaces of the expanded endosperm consisted of both ruptured and intact cells, indicating that some cell separation between cell walls had occurred concurrently with rupturing through cell walls at the time of explosive expansion.

The scutellum and other germ parts do not expand when the kernels are popped. Some starch granules present in the scutellum are gelatinized but others not, and show birefringence with the polarizing microscope. Surprisingly enough, popping does not greatly disorganize the structure of the embryo. Plumule and radicle portions remain topographically intact, although the protoplasmic contents of their cells are altered by the heat-treatment.

The more completely gelatinized starch network in the endosperm stained a typical amyloid blue with dilute iodine in sections of popped milo, dent corn, and popcorn. Staining of ungelatinized or only slightly swollen starch granules varied from blue-black to purple. However, the network of starch gel in dent corn was more finely reticulate or with thinner walls than that of the popped milo and popcorn (compare Fig. 13 with Figs. 11, 12, 15, and 17). Other differences between popped milo, dent corn, and popcorn seem more likely to relate to basic structural distinctions between these grains, such as relative proportions of outer horny outer endosperm vs. the inward, more floury endosperm. The horny endosperm of the milo and popcorn used here completely enclosed a narrow center of floury endosperm, except at the scutellum.

These observations vary in certain respects from those of Carr and Ripley (7). Their photomicrographs show intact cells in the expanded endosperm, but the starch contents of the illustrated cells do not reveal the soap-bubble structure so readily observed in the present studies for the fully expanded endosperm. Carr and Ripley also illustrate what appears to be mainly germ tissue, including the scutellum and the embryonic axis of the germ, as examples of structure in the unpopped kernel. As is evident in Figs. 1 to 3, these tissues are not at all comparable to the endosperm, which is the tissue expanded when the grain is popped.

## DISCUSSION

Occasional statements in the literature that cell structure has been obliterated in the popped popcorn kernel seem to be due to failures to recognize intact cell walls in the expanded endosperm. Possibly the techniques used in preparing microscopic samples were unsuited to the situation. Although considerable swelling of starch granules can occur when they are gelatinized, especially in the formation of the "soap-bubble" reticulum, the escape of steam along with rapid evaporation contributes appreciably to maintenance of cellular integrity. Although some cell rupturing does occur and is necessary for the endosperm to expand explosively, many of the expanded endosperm cells remain clearly recognizable, and cellular dimensions do not appear to be greatly altered. If considered as a sphere, an endosperm cell needs only to increase, slightly over 25% in diameter to double in volume, and this would increase its outer cell-wall surface only by about 60%. Whether the very thin walls of endosperm cells could expand to this extent without rupture seems doubtful. That some walls do rupture is clearly evidenced by the voids in the expanded endosperm, but intervening areas are composed of intact cells that have not swollen extensively because their polyhedral shape is retained.

No attempt has been made here to correlate degree of popping to moisture content and other factors. Obviously, some cereals pop more than others do. Moisture content alone does not appear to have a consistent relation (7), and its influence in popcorn varies with variety, possibly according to kernel structure. Stewart (27), for example, found increases of over 30 times in volume for popped Japan Rice and Amber Rice varieties with moisture between 13.6 and 15.5% but the highest volume increase (24.3 times) for the White Rice var. was obtained at a moisture of 13%. In other studies at this Laboratory (to be published elsewhere), the best popping yields of milo were obtained with samples ranging between 18 and 20% moisture.

It is quite possible that formation of the typical soap-bubble reticulum in popped milo, dent corn, and popcorn relates to combined structural and compositional properties of their starches. Nonwaxy milo starch, for example, gelatinizes at 67° to 75°C.; waxy milo starch gelatinizes at about 5° higher temperature (28). Waxy maize and normal corn starches gelatinize at 63° to 72°C., but high-amylose corn starch is not completely gelatinized by boiling water (29). These Kofler gelation temperatures contrast with those of barley starch (51.5° to 59.5°C.) and wheat starch (58° to 64°C.), which tend to fuse in the popped kernels and do not form the soap-bubble reticulum.

Popping difference also may relate to histological distinctions between different grains (8, 14, 30, 31). These may include the presence or absence of adherent flower parts, such as the flower glume of barley, and the relative strength of pericarp tissues. In addition, the types of endosperm present and their extent of distribution may be quite important with reference to starch properties. For example, the endosperm of flour corn (Mandan) is mostly of the floury or starchy type and only a thin outer shell of horny endosperm is present. Horny endosperm is extensively developed in flint corn, nearly

as much so as in the popcorn kernel, which contains only a small center of floury endosperm (32). Distribution of floury or chalky vs. horny endosperm also varies in different wheats and influences milling qualities (24, 25). Some varieties of barley have mainly a floury endosperm; other varieties are classed as waxy (33). Little is known about the influence of these differences on the popping expansion of different barleys and wheats. Horny endosperm predominates in popcorn, and appears to comprise most of the expanded portion in which cellular integrity is maintained in popped kernels. It seems likely that a similar explanation applies to well-popped milo.

Starch granules from horny and floury endosperms also are morphologically characteristic within each grain. Those from horny endosperm are polygonal and angular, whereas those from floury endosperm are rounded (33). Although the ranges of gelatinization temperature (loss of birefringence) are similar, little seems to be known about differences in their composition which could influence gel properties relative to popping. The starches of different varieties of corn differ in amylose content (33,34). Horny vs. floury endosperms of corn have been manipulated genetically with particular reference to amylose content and starch properties (33). It is reasonable that similar differences should apply to other cereals. Differences in degree of starch gelatinization ranged from the gelatinized soap-bubble structure to ungelatinized starch granules in fully popped milo and popcorn. Frequency of ungelatinized starch granules increased with decrease in popping expansion in these cereals, and this was also characteristic of those cereals in which popping expansion was limited.

It seems unlikely that these differences in behavior of individual starch granules could pertain if heat and moisture conditions were uniform within individual kernels of any one of these cereals. Studies on sweet corn have shown that the lowest moisture content occurs in outer layers near the aleurone (32). It is reasonable that a similar situation applies to mature, dry cereals. That other compositional variation exists within the kernels is clearly evident from ordinary histological examination. The degree of this difference has been more completely revealed by Cox et al. (35) in studies on factors affecting steeping of several corns. They found that after removal of starch from the horny endosperm the remaining proteinaceous network was birefringent and, consequently, highly oriented. In addition, smaller horny-endosperm cells with smaller starch granules embedded in a massive protein matrix lie close to the aleurone, whereas larger starch granules in a less massive protein occur toward the center of the kernel. These findings confirm the explanation earlier advanced by Weatherwax (8) for poppability of flint corns.

Over-all, the horny endosperm contains about twice as much protein as the floury endosperm (35). It would appear that the dense, oriented protein of the horny endosperm not only provides some of the structural barrier so that steam pressure is built for popping, but may even inhibit starch-granule hydration in the smaller cells with more massive protein under the aleurone layer.

These structural and compositional conditions serve to explain popping

expansion differences between different cereals but do not account for the occurrence of individual ungelatinized starch granules within the gelatinized starch matrix within individual endosperm cells. Such ungelatinized granules were observed occasionally here in the soap-bubble network of popped milo and popcorn as well as scattered throughout the endosperm of the partly popped wheat kernels. This suggests some compositional variation also at the starch-granule level. Such variation is not revealed by analytical procedures in which the starch is treated en masse.

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