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Histological Structures of Cooked Rice Grain

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Raw and cooked whole grain sections of milled, medium grain California rice were compared for gross histological and morphological features using fluorescence and scanning electron microscopy (SEM). Cooked and raw grains were compared and the differences were assessed by autofluorescence of the cell walls and correlating fluorescence to SEM images. Milled, raw grains contain fine cracks throughout the endosperm. Cooked grains have wider, more defined cracks, suggesting that they serve as channels for water migration into the grain during cooking. Water penetrates unequally into the grain during cooking: low water penetration produces dense regions with minimal starch gelatinization, and high water penetration produces large voided areas. The voids occur in the transverse orientation of the grain and are the main of cause grain expansion during cooking.

KEYWORDS: Rice; rice histology; cooked rice; grains; fluorescence; microscopy

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major cereal grains of commerce worldwide. Unlike most cereal grains, rice is purchased primarily as milled, raw grains by the consumer, and consumed as a steamed or boiled product. However, as with many foods, consumers have shown more interest in precooked rice products in recent years, particularly in Japan where rice is the staple starch. Precooked rice and rice products have a high water content presenting a new set of problems to the food industry for shipping, shelf life, and storage while maintaining quality. Starch retrogradation (*1*), another quality parameter, occurs after cooking and with more rapidity under refrigerated conditions. Refrigerated storage is often the method of choice for prolonging shelf life of high water content food products.

Texture is yet another quality parameter of cooked rice and is traditionally determined using destructive physicochemical measurements (*2*, *3*). Nondestructive texture determination of cooked rice was accomplished using NMR microimaging, a spectral technique that can be correlated to texture (*4*, *5*), where the grains were shown to contain internal voids. The voids might have occurred because of the presence of cracks in the milled, raw grains. Microscopy of individual grains was used to identify structure and histology contributing to textural properties in previous studies. Wood et al. (*6*) embedded grains in glycol methacrylate resin, which allows the embedded material to be stained readily with biological dyes, and used light microscopy to study sections of rice grains. Nakano et al. (*7*) studied complete cryosections of cooked rice grains. In both of the previous studies, the sections included too small an area or were of poor quality and were inadequate for the evaluation of overall histology of cooked rice. In the present work, SEM images were correlated with fluorescence images made from complete, high quality sections of cooked rice grains. The method of Ogawa which was developed for the visualization of three-dimensional component distribution (*8*, *9*) and morphological structure (*10*) in rice grains was modified in the current work.

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MATERIALS AND METHODS

Materials. Milled, nonwaxy, medium grain rice (cv. unknown, Enriched Calrose Rice, packed by Safeway Inc., Pleasanton, CA) was purchased at a local supermarket in Albany, CA in 2002.

Cooking. Milled, raw grains (72 g) were placed in a microwavable rice cooker (Apex rice cooker, Daiya Co., Japan) in 100 mL of water and soaked at room temperature for 20 min. The rice cooker, soaked rice, and water were then placed into a microwave oven (500 W, Kenmore, Sears, Roebuck and Co., Hoffman Estates, IL) on "high" for 7 min. After cooking, the rice grains were allowed to sit for 15 min before preparation for microscopy. The cooking method was that recommended by the manufacturer of the rice cooker with a slight modification which accounted for the small quantity of rice.

Section Preparation. Whole milled, raw (raw) and milled, cooked (cooked) grains of rice were dehydrated in a graded ethanol series (30% × 2, 40%, 50%, 60%, 70%, 80%, 90%, 95% × 2, 100% × 2) and infiltrated with xylene at 21 °C, 1 day per step. The grains were then transferred to melted paraffin at 70 °C (melting range: 66−68 °C) and allowed to infiltrate for 1 day. The resulting paraffinized grains were placed into embedding molds with additional paraffin and chilled to allow for hardening of the paraffin. Before the paraffin was completely hardened, the microtome chuck was gently placed on top of the mold to secure the specimen for sectioning.

Embedded rice grains were sectioned, at ambient temperature, using a rotary microtome (CM 3000 cryostat, Leica, Germany) equipped with disposable (Leica model 819) blades. Each grain was trimmed until the desired portion was exposed and the adhesive tape method (*8*) was employed to collect 10-μm-thick sections. Adhesive tape (Toshiba Machine Co., Ltd., Japan), consisting of a polyester base which is transparent to visible light, coated with a solvent-type acrylic resin adhesive, was used to collect sections (*11*). The tape+section was affixed to a glass slide with the specimen side facing up using a thin strip of adhesive tape and deparaffinized in xylene for 3 h.

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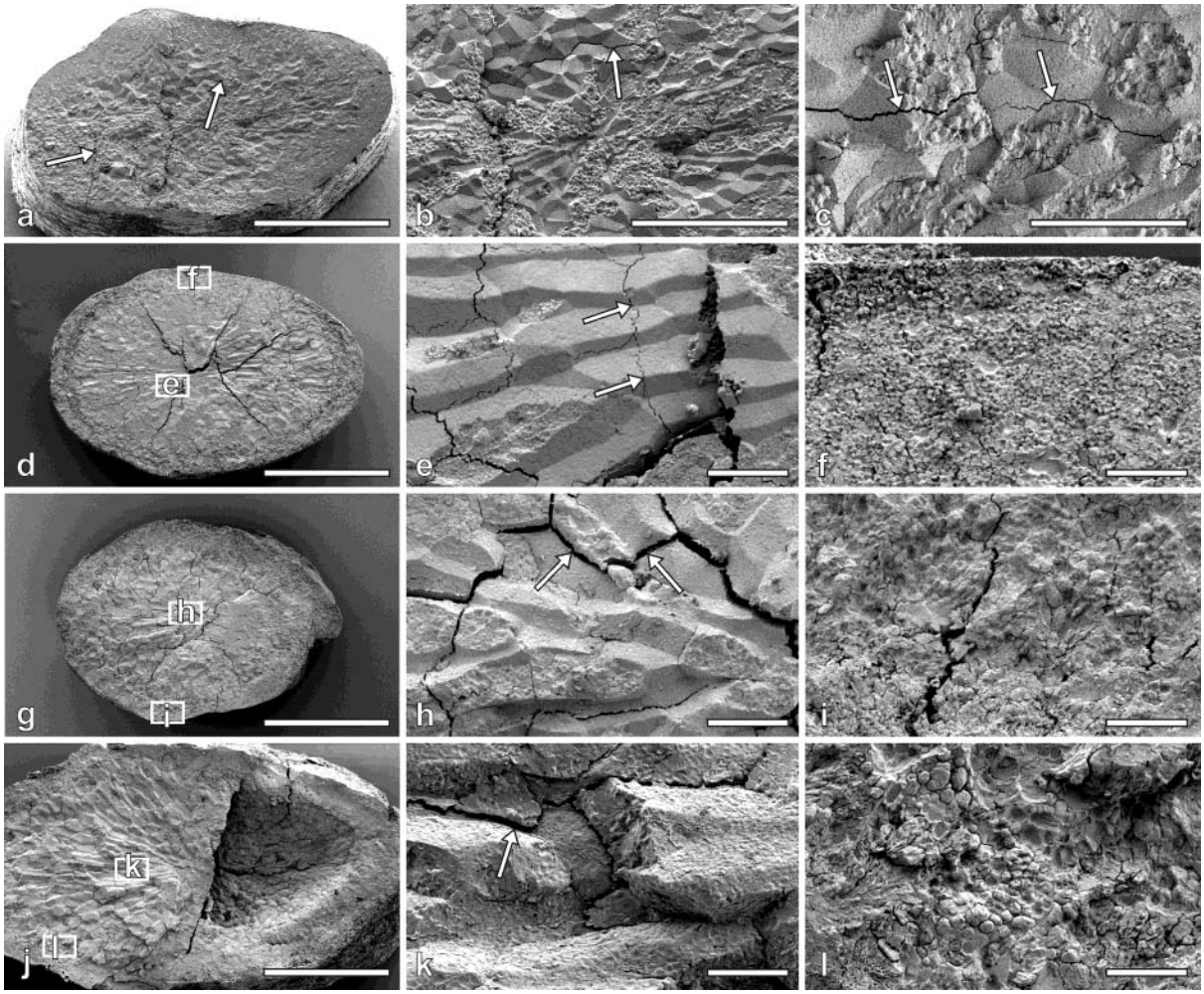

Figure 1. Scanning electron micrographs of cross sections of raw and cooked kernels. (a–c) Dry-fractured raw grains showing that the cracks in the grain are not artifacts due to specimen preparation for microscopy. Cross fracture showing the entire milled kernel (a); higher magnifications (b, c) showing both gross and finer cracks. Cracks (arrows) are probably due to dehydration of the grains during maturation or ripening. (d–f) Raw grain, dehydrated in ethanol, showing large and small cracks that have become more defined as a result of the ethanol dehydration. Interior (e) and peripheral (f) areas of a raw grain. (g–i) Cooked grain showing an area without voids containing fine cracks. The areas in rectangles are shown in (e) and (f). Interior (h) and peripheral (i) areas of a cooked grain. (j–l) Cooked grain showing an area containing a void. Areas in rectangles are shown in (k) and (l). Interior (k) and peripheral (l) areas of a cooked grain. Scale bars: 1 mm (a, d, g, j); 500 μm (b); 200 μm (c); 50 μm (e, f, h, i, k, l). Arrows point to cracks.

Scanning Electron Microscopy. Raw grains were dry-fractured or were dehydrated and then fractured using a razor blade. Cooked rice grains were dehydrated as for light microscopy through 100% ethanol and cryofractured in liquid nitrogen (*12*), because cooked rice grains were soft and sticky. All samples, except those that were dry-fractured, were critical point dried (Tousimis Autosamdri 815, Tousimis, Rockville, MD). Dry-fractured and critical point dried specimens were mounted onto aluminum specimen stubs using a graphite-epoxy mixture or double-coated carbon tabs (Ted Pella, Redding, CA). Whole grain sections were collected onto slides, deparaffinized, and air-dried, and then removed from the slide and attached to an aluminum specimen stub with double-coated carbon tabs. All SEM samples were sputter coated with gold−palladium using a Denton Desk II sputter coater (Denton Vacuum, Co., Moorestown, NJ) and photographed using a field emission scanning electron microscope (S-4700, Hitachi, Japan).

Light Microscopy. Deparaffinized sections were mounted in immersion oil (Type A, Cargille Laboratories, Inc., Cedar Grove, NJ) and coverslipped. The mounted sections were observed and photographed in a Zeiss Universal Research compound microscope (Zeiss, Germany) equipped with an HBO 50W mercury arc lamp and the IIIRS filter cube for fluorescence. The UV filter set (BP365, excitation filter; FT395, beam splitter; LP397 nm, barrier filter) and a 10× objective lens (DplanApo 10 UV 0.4, 160/0.17, Olympus, Japan) were used for autofluorescence. The images were captured by a commercially available digital camera (Coolpix 950, Nikon, Japan), which was mounted on the microscope using an adaptor lens (Coolpix MDC lens, Nikon, Japan). Focus, exposure, and aperture parameters of the camera were selected manually. The images were captured as 24-bit RGB and were converted to 8-bit gray scale images. Image processing was performed with a commercially available graphics package (Corel PhotoPaint 10, Corel Co., Canada).

RESULTS AND DISCUSSION

Rice Grain Fractures − SEM. Figure 1 shows SEM cross sections of raw and cooked grains. **Figure 1a−c** shows dry-fractures of whole cross sections through single, raw grains. The micrographs show that the cracks are present in the starchy endosperm in a dry-fractured sample. The cracks probably occur as the grain loses water through the course of maturation. **Figure 1d−f** shows raw grains that were dehydrated through a graded series of ethanol and then fractured. Following ethanol dehydration of the sample, the large cracks have become more evident (**Figure 1d**) than those in the dry-fractured sample. Fine cracks appear jagged in the raw grain (**Figure 1e**, arrows). Cooked

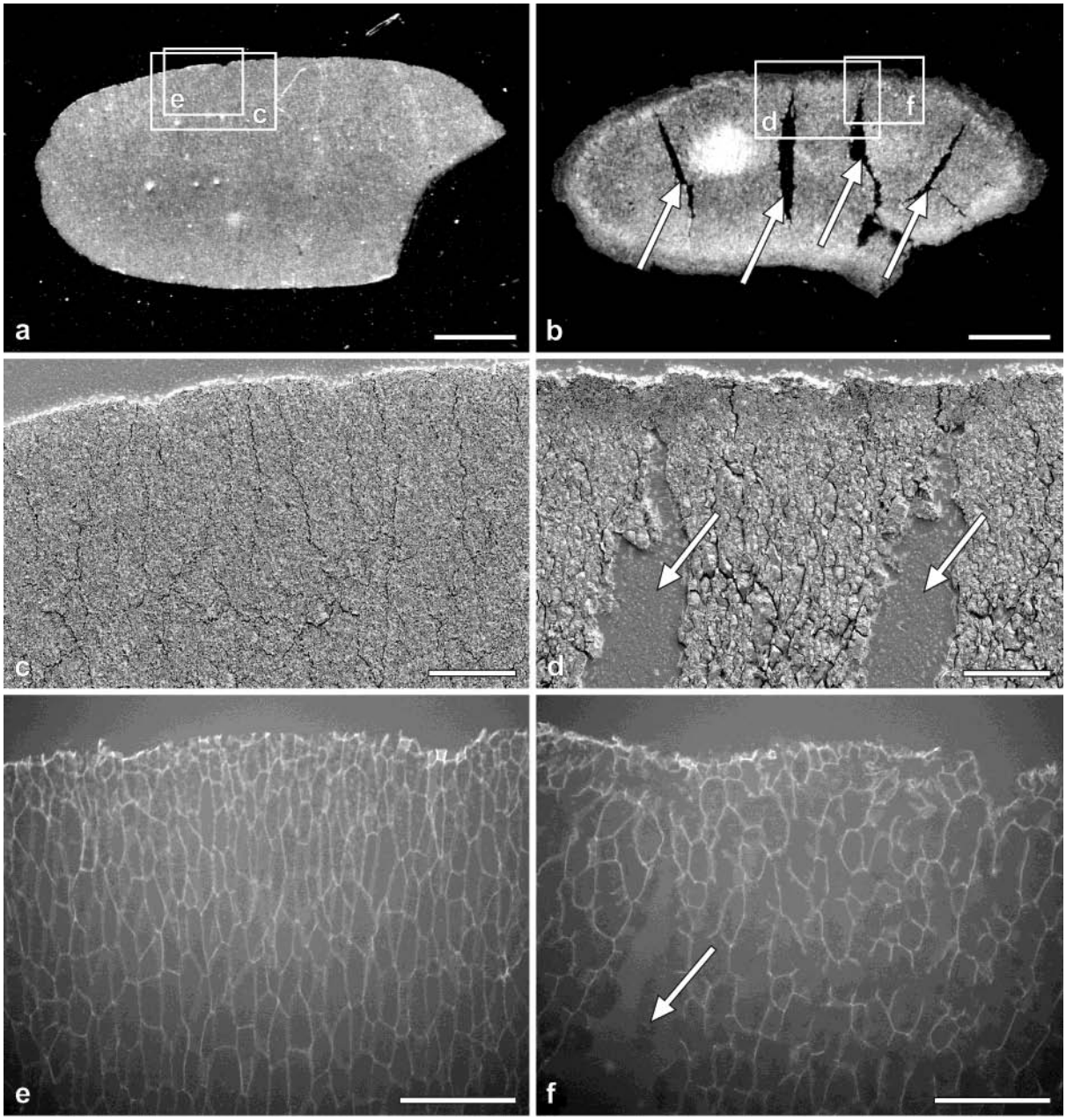

Figure 2. Longitudinal sections of raw (a, c, e) and cooked (b, d, f) rice grains. The rectangles in a and b indicate areas shown in higher magnification in the subsequent micrographs. Light micrograph of a section of a raw grain (a) showing the intact grain and the obvious absence of the outer bran layer and the germ (embryo). Light micrograph of a section of a cooked grain showing voids (arrows) in the transverse orientation of the grain (b). Scanning electron micrograph (SEM) of the periphery of a section adjacent to (a) of a raw grain (c). SEM of the periphery of a section adjacent to (b) of a milled, cooked grain (d) showing voids (arrows) and the surrounding tissue containing swollen, gelatinized starch granules. Note the smooth appearance of the tissue on the periphery is different than the more textured, or rough interior. Autofluorescence (365 nm excitation) image of the section shown in (a) of milled, raw grain showing intact cell walls (e) and of the section shown in (b) of milled, cooked grain (f) showing the disruption of the cell walls and a void (arrow). Magnification bar: a–b, 1 mm; c–f, 200 μm.

grains (**Figure 1g−l**) also contain cracks in the starchy endosperm which appear similar to those in the raw grains. However, the fine cracks in the cooked grain are wider and more defined (arrows, **Figure 1h,k**) than cracks in the raw grain. The differences between raw and cooked grains in microstructure of the cracks suggest that they serve as channels for the flow of water into the grain during cooking. The periphery of a cooked grain contains starch granules which remain evident as individual structures that have increased in diameter (**Figure 1l**) compared to the raw state (**Figure 1b,c,f**), which contains unmodified starch granules throughout. The size increase of the starch granules in cooked rice is due to the uptake of water, consequent swelling, and gelatinization, giving a "melted" appearance to grain periphery. Two types of structures are evident in the cooked grains: those where the starchy endosperm remains intact (**Figure 1g−i**) and those containing voids in the central endosperm (**Figure 1j**). The microenvironment of the grain periphery is vastly different from that of the grain center. The peripheral cells are smaller in diameter and contain more protein than those in the center of the grain. Additionally, a buildup of pressure occurs in the center of the grain that does not happen at the periphery. Therefore, the periphery remains intact during cooking, whereas the grain center contains both intact and voided areas.

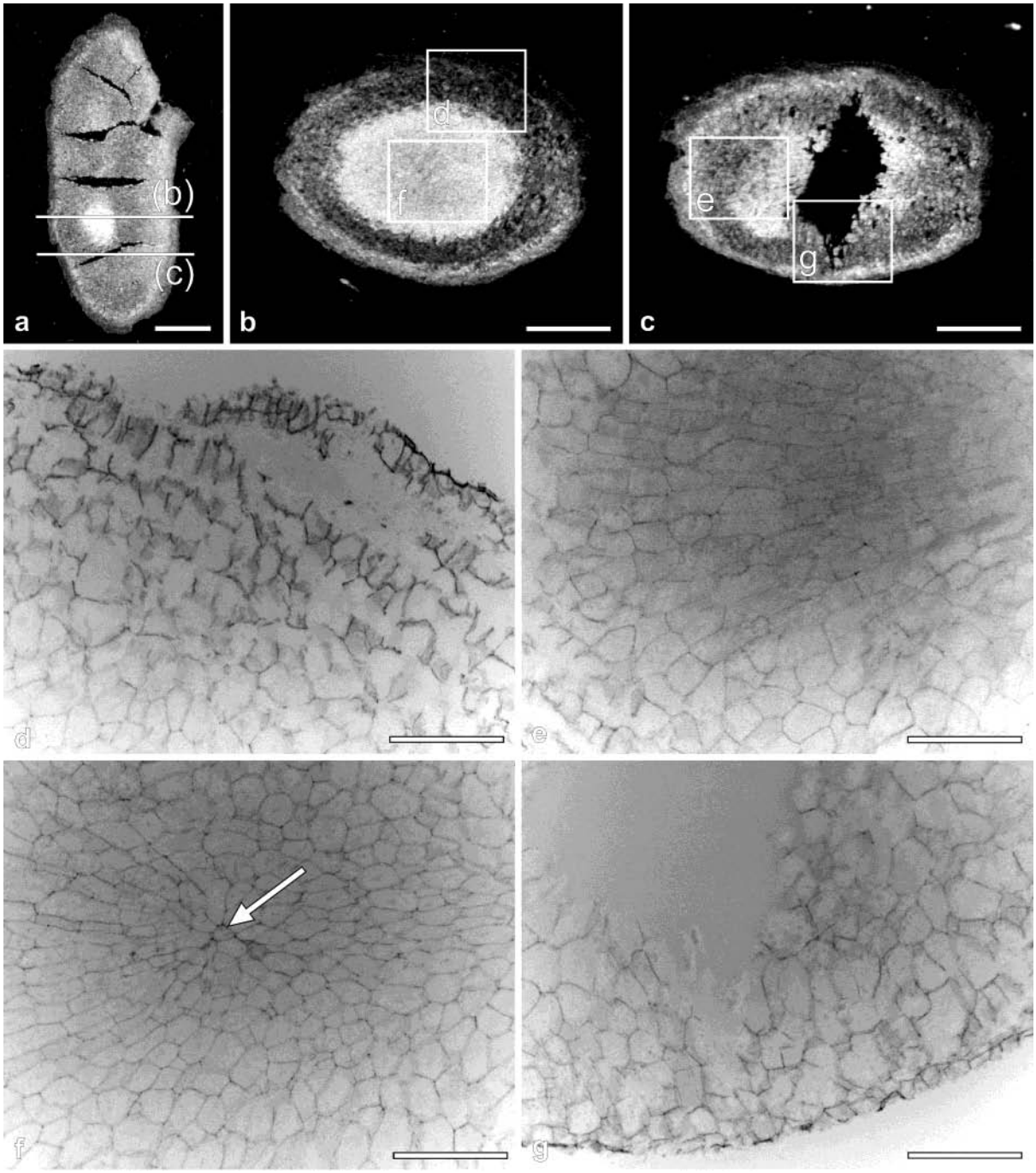

Figure 3. Light micrographs of sections of cooked rice grain. Longitudinal section of a whole grain (a). Cross sections of an optically dense area (b) and a "void" area (c). Autofluorescent regions of the rectangles areas in b (d, f) and in c (e, g). Arrow in f indicates the center of the grain. Magnification bars: a–c, 1 mm; d–g, 200 μm.

Rice Grain Sections − Light Microscopy. Figure 2 shows longitudinal sections of raw (**Figure 2a,c,e**) and cooked (**Figure 2b,d,f**) rice grains. The longitudinal sections clearly show that the voids occur in the transverse orientation of the grain (arrows, **Figure 2b**). The voids account for most of the deformation and swelling of the grain during cooking and are probably the result of rapid pressure buildup (steaming) and subsequent expansion or localized explosion (**Figure 1c**) within the grain. Voids or "internal hollows" in cooked rice grains were also reported by Horigane et al. (*4*, *5*) using NMR microimaging. Scanning electron micrographs of serial sections show the structure and surface characteristics of raw [**Figure 2c**, serial section of **Figure 2a** (enlargement of inset c)] and cooked [**Figure 2d**, serial section of **Figure 2b** (enlargement of inset d)] grain sections. The peripheral layer of the cooked grain has a smooth surface creating a "melted" or "fused" appearance (**Figure 2d**) that is not evident in the raw grain (**Figure 2c**). Cell disruption with cooking can be determined by taking advantage of the autofluorescence characteristics of phenolic compounds in plant cell walls (**Figure 3e,f**). Phenolic compounds fluoresce when exposed to UV light at about 365 nm (*13*). A section of a raw grain [**Figure 1e**, serial section of 1a (enlargement of inset e)] shows the autofluorescent cell walls, suggesting intact cells which are elongated in the transverse orientation of the grain. A cooked grain section [**Figure 1f**, serial section of 1b (enlargement of inset f)] shows broken cell walls and a void (arrow), indicating that the cells have expanded to the point where the cell wall was ruptured.

Cell Damage of Cooked Rice Grain. Figure 3 shows longitudinal (**Figure 3a**) and cross sections (**Figure 3b,c**) and cell wall distribution of cooked rice grains. **Figure 3a** illustrates the relative positions of the cross sections shown in **Figure 3b** (intact portion) and **3c** (void portion). Portions of the sections have differences in optical density (white center in **Figure 1a** has a higher density than that of the surrounding tissue). The variations in optical characteristics might be caused by differences in the degree of starch gelatinization (*14*), suggesting heterogeneity of the structural elements of the cooked grain, particularly in visible light. **Figures 3d−f** are autofluorescent images showing the areas indicated in rectangles in **Figure 3b,c**. Simple image processing steps were carried out to invert and enhance the contrast in the images to increase the resolution. The outermost layer of cells in the cooked grain has been completely disrupted (**Figure 3d**), and the cells beneath the surface have disrupted cell walls. The central starchy endosperm of the cooked grain has cells with minimal disruption (**Figure 3e**) and cells without evidence of disruption (**Figure 3f**). The cells in the optically dense area shown in **Figure 3f** are not disrupted, thus, as suggested by Juliano (*14*), the intact cells differ in starch gelatinization characteristics due to the limited accessibility to moisture compared to the surrounding tissues. The cooked grain also contains "voids" (**Figure 3g**) and the cells along the perimeter of the void have been greatly disrupted.

Water penetrates unequally into the rice grain during cooking. The interaction of water with the various regions of the grain can be interpreted in the micrographs in terms of the amount of visible cell wall disruption. Cell wall disruption (**Figure 3f**) is lacking in the optically dense regions (**Figure 3a,b**) due to limited water interaction with the cells. Cells have undergone disruption along the periphery of the grain (**Figure 3b−d**) and in areas where voids occur (**Figure 3a,c,g**). The voids most likely result from pressure buildup within the grain due to superheating of water which then turns to steam. The steam cannot escape; therefore, the grain ruptures to release the pressure, creating a tear or void (**Figure 3a,c,g**) in the transverse direction of the grain (**Figure 3a**). Thus, cell walls tend to be damaged to the greatest extent where water penetrates into the grain. During the cooking process, various cellular components, such as starch, nonstarch carbohydrates, lipids, and proteins, leach into the cooking water, turning the water into a viscous liquid. The amount of leached material depends on the amount of cell wall disruption occurring during cooking. As cooking progresses, the water evaporates and the leached components precipitate, creating a film that covers the surface of the rice grains. The film contributes to the texture and eating quality of rice (*15*). Thus, the dissolved components and, therefore, cell disruption play significant roles in the eating quality of rice.

CONCLUSIONS

Milled, raw grains contain fine cracks throughout the endosperm. Once the grains are cooked, the cracks are wider and more defined, suggesting that the cracks serve as microchannels for water migration into the grain during cooking. The dense regions with minimal starch gelatinization in cooked grains are evidently areas with low water penetration. The voided regions are areas of high water penetration; thus, the cracks or absence of cracks create the unequal uptake of water into the grain during cooking. Increased water penetration during cooking causes more cell wall disruption.

Safety. Xylene is highly flammable and was used in a fume hood.

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