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Fibrillar Microfilaments Associated with a High-Amylose Rice, Goami 2, a Mutant of Ilpumbyeo, a High-Quality Japonica Rice

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The ultrastructure of cooked and malt-treated cooked rice of Ilpumbyeo (IP) and its mutant Goami 2 (G2), which have extreme contrasts in physicochemical properties, cooking quality, and ultrastructural characteristics in raw grains (*1*, *2*), was compared. In cooked rice of IP, starch granules in endosperm cells were evenly coalesced, appearing as homogeneously smooth sheetlike matrix and/or globules, whereas those in G2 were a heterogeneously coarse matrix in which a novel structural feature, the microfilaments, was embedded. In malt-treated cooked rice of IP, most starch was hydrolyzed by the malt enzymes, appearing as empty vacuoles surrounded by the cell wall, whereas that in G2 was highly resistant to malt treatment, remaining as distinct structural features, the malt-resistant compound starch granules. The property of G2's compound starch granules, which are tolerant of mechanical and chemical treatments thereby retaining their structural integrity (*2*) and of cooking and malt treatment thereby retaining their physical hardness, appears to play a major role in determining the quality of cooked rice of G2.

KEYWORDS: Microfilaments; ultrastructure; TEM; cooked rice; malt-treated rice; resistant starch; high-amylose rice; microfilaments; compound starch granules

INTRODUCTION

Recently, it has been reported that a new rice variety, Goami 2 (G2) (formally called Suweon 464), developed by mutation breeding via *N*-methyl-*N*-nitrosourea (MNU) treatment of Ilpumbyeo (IP), a high-quality japonica rice, has extreme contrasts in every aspect of physicochemical properties, cooking quality, ultrastructure of starch endosperm cells in situ, and isolated starch granules to those of its mother variety, IP (*1*, *2*). Unlike most cereal starches, including rice, G2 had B-type crystallinity and markedly lower proportion of short chains in the distribution of a glucan-chain fraction of debranched starch, which were apparently the reflection of its unusually high-amylose content and would contribute to its unsuitability for ordinary cooked rice. Indeed, the quality of cooked rice of G2 was so poor that it was almost unacceptable for an ordinary meal due primarily to its lower swelling power, poor gelatinization, higher hardness, and less stickiness as compared with other ordinary rice (*1*). Although these properties may not be suitable for ordinary cooked rice, however, G2 has, from the nutritional point of view, promising properties as an excellent candidate for other processed healthy food products based on its unusually higher contents of fiber, protein, and lipids (*1*).

Ultrastructural comparisons of starch endosperm cells in in situ, fractured whole grains, and isolated starch granules between IP and G2 revealed striking differences that could account for their extreme contrasts in cooking quality and physicochemical properties (*2*). For example, most of compound starch granules in G2 were enclosed within a sac-like structure that was tolerant of severe physical as well as chemical treatments involved in fracturing and starch isolation processes, thereby preventing the release of individual starch granules clustered within them, whereas those in IP were readily split and/or dissociated completely during the treatments releasing the individuals within them freely (*2*). It is obvious, therefore, that such sac-enclosing compound starch granules in G2 would also limit the entrance of water into the granules and subsequent absorption, leading them to poor swelling, incomplete gelatinization, and finally to poor quality of cooked rice.

Recently, our laboratory has initiated studies on the utilization of G2 in the production of a variety of processed foods other than ordinary cooked rice, including Shickhae, a traditional rice food in Korea. Shickhae is a malt-treated cooked-rice beverage that has been highly popular and consumed by the general public nationwide for hundreds of years. Preliminary studies, however, indicated that G2 was unsuitable for Shickhae due primarily to the inability of amylolytic enzymes in the malt to convert its starch sufficiently to glucose and maltose. Cooked rice grains of G2 following the malt treatment retained their hardness to a

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degree that is unacceptable for the beverage, whereas those of IP were softened ideally for it.

Since the extreme contrasts in physicochemical properties and cooked rice qualities between IP and G2 (*1*) were clearly reflected in the ultrastructure of starch endosperm cells in in situ and fractured as well as isolated starch granules of uncooked raw grains (*2*), studies were undertaken to investigate how these contrasting differences between the two would be expressed in the ultrastructure after they were cooked for ordinary consumption and treated with the malt for the beverage, Shickhae. This paper presents the results of thin-section electron microscopy (TEM) of cooked and malt-treated cooked grains of IP and G2 rice, demonstrating striking differences accountable for the reflection of their physicochemical as well as ultrastructural differences shown in uncooked raw grains (*1*, *2*). The fibrillar microfilaments embedded in the coarsely gelatinized starch matrix present in the cooked rice of G2, which were resistant to malt treatment, appear to be the major determinant in governing its quality of cooked rice. The fibrillar microfilaments of G2 or similar structures have not previously been reported to occur in any rice variety.

MATERIALS AND METHODS

Rice. The source of milled, raw grains of the mutant, G2, and its mother variety, IP, was the same as those used for the previous studies (*1*, *2*). The rice plants were cultivated in a rice experimental field at the Institute of Crop Science, Suwon, Korea, and the crops harvested during the 2001 to 2003 growing seasons were used.

Cooking. Milled raw grains of each type of rice (200 g) were rinsed three times with tap water and presoaked for 2 h at room temperature. Water was then added to the rice to give a volume ratio of 1:1 in an electric rice cooker (LG rice cooker, Korea). After being cooked, rice grains were allowed to cool for 30 min at room temperature before they were prepared for electron microscopy.

Malt and Shickhae Preparation. The malt used in this study was prepared with barley (*Hordeum vulgare* L.) by the procedure used by Yun et al. (*3*), which showed the highest levels of amylolytic and β-glucanase activities. Saccharification of the cooked rice for Shickhae preparation was carried out by a traditional method commonly used by the public (*4*). Briefly, the malt extract diluted 1000× with distilled water was added to the cooked rice in a ratio of 5 (malt) to 1 (rice) in the electric rice cooker at the warm setting overnight.

Hardness Measurement of Cooked and Malted Rice. Hardness of cooked and malted rice grains from IP and G2 was measured with a texture analyzer (TA-XT2, Stable microsystems, Godalming, England) by placing individual grains on the base plate of the analyzer at room temperature. A force in compression with a force versus time program was used with a test speed of 2 mm/s, distance of 75%, and a cylinder plunger that was 2 mm in diameter.

TEM. Individual grains of cooked and malted IP and G2 rice were cut transversely with a razor blade in the midregion and one or two 1.0∼1.5 mm thick slices were removed. Each slice, which was semicircular in shape, was further cut into a number of tissue pieces approximately 1∼2 mm^2, each of which included the peripheral and central regions of endosperm. The tissue pieces were prepared for TEM by the method used for the previous study (*2*). Briefly stated, the tissues were fixed in a modified Karnovsky's fixative (*5*), post-fixed in 2% osmium tetroxide, en bloc stained in aqueous 2% uranyl acetate, dehydrated in an ethanol series from 30 to 100%, and embedded in Spurr's low-viscosity embedding medium (*6*). Thin sections of embedded tissue blocks were cut with a diamond knife and were stained with 2% aqueous uranyl acetate followed by lead nitrate. Sections were viewed and photographed by Zeiz LEO 906 TEM at 80 kV. Like in the case of raw grains (*2*), the quality of sections was poor, producing numerous folds and torn areas, but the ribbons sectioned slightly thicker produced better results.

Table 1. Hardness (*g*) of Cooked and Malt-Treated Rice Grains from IP and G2

variety	cooked rice	malt-treated rice
IP	1657.68 ± 107.20[a]	89.80 ± 4.46
G2	2425.00 ± 102.48	540.60 ± 7.16

[a] Measurements were made in triplicate, and in each time, 20 individual grains were measured. The results are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Hardness of Cooked and Malt-Treated Rice Grains of IP and G2. Individual grains of both cooked and malt-treated rice of G2 were much harder than those of IP (**Table 1**). The hardness of G2 grains in cooked and malt-treated rice was about 1.5 and 6 times higher than those of IP, respectively (**Table 1**), indicating that G2 cooked rice was extremely resistant to the malt treatment, whereas IP cooked rice was highly susceptible to it. These wide differences in hardness between IP and G2 cooked and malt-treated rice are apparently the reflection of the differences in physicochemical as well as basic component composition (*1*) and also in the ultrastructure of starch granules (*2*).

Cooked Rice Ultrastructure. In cooked rice grains of both IP and G2, the major cellular components of starch endosperm cells such as the starch, protein bodies, and cell wall remained morphologically identifiable (**Figures 1** and **2**) based on their general morphology, cellular location, and electron density, which were comparable to those observed in row grains (*2*, *7*, *8*). Individuality of starch granules was, however, not distinct (**Figures 1** and **2**) due apparently to their coalescence into each other during the processes involved in cooking and retrogradation, such as swelling, heating, gelatinization, and cooling.

IP. Cooked starch in IP appeared as homogeneously smooth, glossy, sheetlike matrices of various sizes and shapes (**Figures 1** and **4A**). In the cell periphery near the cell wall, the starches were globulized into smaller sizes apparently formed by being detached from those internally located larger sheetlike forms (**Figure 1**). Smaller globules were usually circular in shape and surrounded by electron-lucent interglobular spaces, which were apparently the air spaces (**Figures 1** and **4A**). It is suggested that some of these air spaces could be an artifact produced by a viscoelastic function of the gelatinized starch during the processes of specimen preparation for microscopy, such as the slicing and dissecting of the softened cooked rice grains into smaller pieces.

In cells near the surface of a grain, presumably subaleuron starch endosperm cells or those nearby, more of the globular forms of starch were observed, whereas for those in the central region, extremely large sheetlike forms occupying most of cell dimensions were common (**Figure 1**). Regardless of globular or sheetlike forms, the basic morphology of the matrices of IP starch, homogeneously smooth and amorphous in nature, was the same (**Figure 1**). Protein bodies were, like in uncooked raw grains (*2*, *9*), usually located near the cell wall associated with the remnants of cytoplasm remaining after endosperm maturation (**Figures 1** and **9A**). Spherical prolamine protein bodies (PB I) retained their structural integrity after being cooked, but irregularly shaped glutelin protein bodies (PB II) lost some of their morphological characteristic (**Figures 1** and **9A**). Apparently, PB IIs were partially dissociated or structurally altered during cooking and were merged with other cytoplasmic remnants appearing as electron-dense stripes along the cell wall (**Figures 1** and **9A**). It agrees with previous reports that PB I, but not PB II, retained its structural integrity not only after being

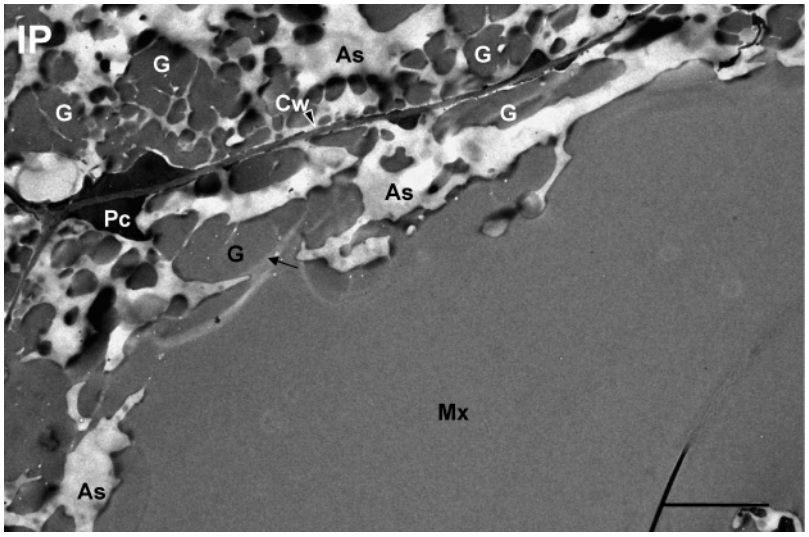

Figure 1. Low magnification view of endosperm cells from cooked rice of IP showing homogeneously smooth and glossy sheetlike starch matrix (Mx) and globules (G) of various sizes and shapes (scale bar = 5 μm). The globules are more common near the cell wall (Cw) and are surrounded by electron–lucent air spaces (As). Some globules are connected to the centrally located large sheetlike starch matrix (unlabeled arrow), indicating that the former is detached from the latter by viscoelastic function of gelatinized starch. Protein bodies and associated cytoplasmic remnants (Pc) occurring near the cell wall appear extremely electron-dense, and therefore, structural details are not clearly shown because the micrograph was focused on the starch matrix. Refer to Figure 9A for more details of protein bodies and Pc.

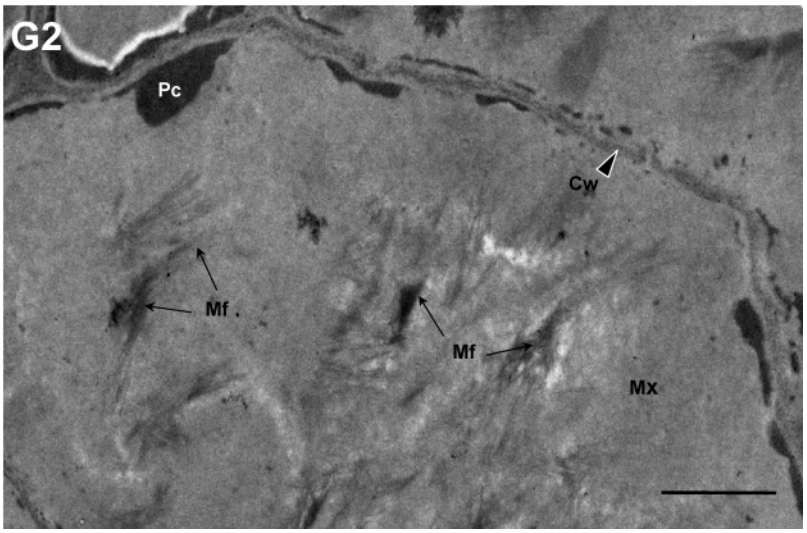

Figure 2. Low magnification view of cooked rice endosperm cells from G2 showing heterogeneously coarse and grainy sheetlike starch matrix (Mx) extending throughout the cells without the interruption of air spaces (scale bar = 2 μm). The bundles of fibrillar microfilaments (Mf) are scattered randomly in the matrix. Cw = cell wall and Pc = protein bodies and associated cytoplasmic remnants.

cooked but also after raw or cooked rice was fed to monogastric animals (*10*−*12*).

G2. In contrast to IP, cooked starch in endosperm cells of G2 was not globulized in any region of a cell or grain but appeared as an evenly flat sheetlike matrix extending throughout the cells without the interruption of air spaces (**Figure 2**). This apparently is the result of viscoelastic property lacking in cooked rice of G2. The protein bodies and associated cytoplasmic remnants that appeared as extremely electron-dense strips or patches, like those in IP, occurred near the cell wall and also sporadically in the starch matrix (**Figure 2**). The coalescence of starch granules in the grain, like in IP, must have occurred during cooking since no individual starch granules were discernible. However, the matrix of the sheets in G2 starch had its own morphological characteristics by being somewhat coarse and grainy as compared with the smooth and amorphous nature of IP starch matrix (**Figure 2**). In addition, G2 starch revealed the presence of a novel structural feature, which was absent in IP, the microfilaments (Mfs) of approximately 15∼20 nm in width and undetermined length (**Figures 2** and **3**). The Mfs, which were embedded in the sheet of the coarse and grainy starch matrix, were either straight or slightly curved rods occurring usually as bundles of various sizes (**Figure 3**). No particular regions in a grain or within an individual cell for the location of the Mfs were noticed. They were random in distribution, but several or more of the bundles located as a group in certain areas were not uncommon (**Figures 2** and **3**).

As indicated, the major ultrastructural manifestation of the differences between cooked rice of IP and G2 was the appearance of the starch matrixes, the homogeneously smooth and glossy versus heterogeneously coarse and grainy, and the absence and presence of the Mfs (**Figures 1, 2,** and **4A,B**), respectively. It should be noted, however, that the coarseness and graininess and the presence of Mfs in G2 were not evident

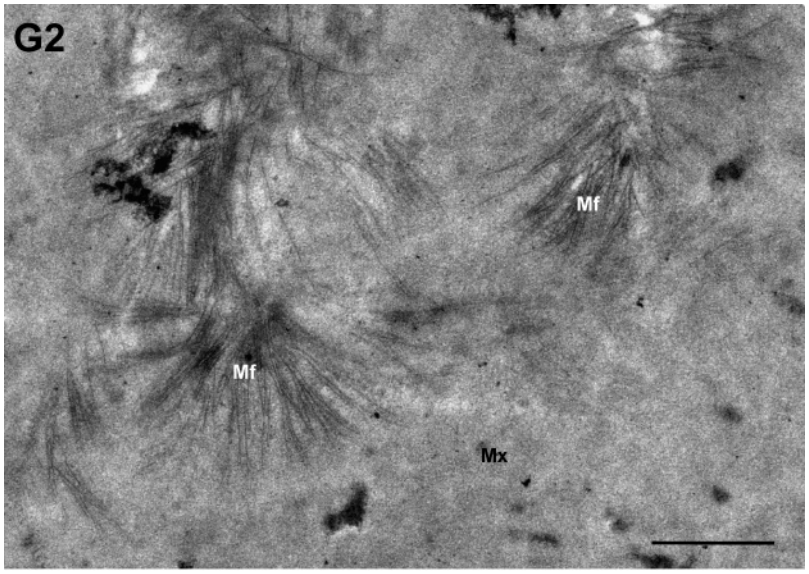

Figure 3. Higher magnification view of the fibrillar microfilaments (Mf) embedded in coarse and grainy starch matrix (Mx) from a grain of cooked rice of G2 (scale bar = 1 μm). The filaments are straight or slightly curved being approximately 10–15 nm in diameter with undetermined length.

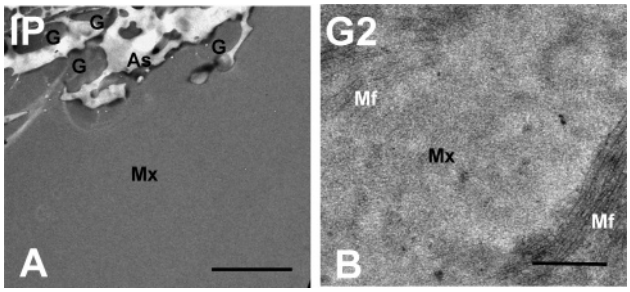

Figure 4. Structural comparison of starch matrices between cooked rices of IP (**A**) and G2 (**B**). (**A**) From IP. Starch appears as homogeneously smooth and glossy sheetlike matrix (Mx) (Scale bar = 5 μm). The upper left corner shows small globular forms (G) surrounded by air space (As). (**B**) From G2. Starch appears as heterogeneously coarse and grainy sheetlike matrix (Mx) without the interruption of air space (scale bar = 0.5 μm and Mf = fibrillar microfilaments).

in uncooked raw rice grains (*2*) but occurred when the grains were cooked. It is apparent, therefore, that the processes involved in cooking such as hydration, swelling, heating, gelatinization, and subsequent retrogradation should be responsible for their appearances.

Starch gelatinization, the most important determinant of texture in a cooked rice product, is a phenomenon of the irreversible collapse of molecular order within a starch granule when heated in excess of water at a certain temperature (*13*). The ultrastructure of cooked rice grains of IP and G2, presented in this paper, is, therefore, considered to be the study of irreversibly swollen, gelatinized, and retrogradated starch gels confined within a whole grain form. Since milled rice, the most commonly eaten form, is made primarily of starch (amylose and amylopectin), making up about 90% of dry weight (*14*), its gelatinization and subsequent retrogradation are the two major determinants in controlling the quality of cooked rice. However, milled rice also contains, as a minor constitute, protein, lipids, minerals, and nonstarch polysaccharides. Although present in smaller amounts, lipids, especially in cereal starches, have been demonstrated to generate complex molecules as a result of intra- and/or intermolecular interactions with starch components during the cooking of whole rice grain (*15*, *16*). These starch−lipid complexes, in addition to the amylose−amylopectin ratio, have been shown to greatly influence on the properties of gelatinization and retrogradation of rice (*17*−*19*).

In previous studies, it has shown that IP and G2 rice have extreme contrasts not only in the amylose−amylopectin ratio but also in the basic component composition and physicochemical as well as texture properties of cooked rice (*1*). G2, a high-amylose rice, has an unusual B-type X-ray diffraction pattern as a rice starch and lower relative crystallinity, whereas IP, a low-amylose rice, has a typical A-type X-ray diffraction pattern like those of most ordinary japonica rices (*20*, *21*) and higher relative crystallinity (*1*). In the basic component composition, G2 has a higher value in every native component analyzed including protein, lipid, dietary fibers, and ashes. Among these, it should be noted that the contents of lipids and dietary fibers in G2 are unusually higher, approximately 4 and 2 times, respectively, than those in IP (*1*).

Ultrastructural comparison between cooked rice of IP and G2, presented in this paper, also demonstrated striking differences, revealing that the each has its own characteristic morphological feature, which is apparently the reflection of physicochemical as well as basic component compositional differences existing between the two. The homogeneously amorphous, smooth matrix shown in cooked rice starch of IP seems to have resulted from a well-balanced amylose−amylopectin ratio of a low-amylose rice of IP (*20*), which would lead to an ideal gelatinization and retrogradation for a good quality of cooked rice. It appears that the amount of other basic components, such as protein, lipids, and fibers, in IP is not significant enough to interfere with the glossy or smoothness of the starch matrix of cooked rice. It is of interest to find out whether the rice classified as low-amylose rice (*20*) having similar ranges of the amylose−amylopectin ratio and other basic components to those of IP would produce similar ultrastructural features to that shown in cooked rice of IP. If it is the case, the homogeneously smooth appearance of cooked rice may then represent an ultrastructural manifestation of well-gelatinized starch matrix of low-amylose rices that would lead to a good quality of cooked rice.

On the contrary, the heterogeneously coarse and grainy matrix in which the bundles of Mfs were embedded (**Figures 2, 3,** and **4B**) shown in cooked rice of G2 appears to have resulted from an ill-balanced amylose−amylopectin ratio of a high-

amylose rice of G2, which would lead to poor gelatinization and retrogradation and thus to unsuitable quality for consumption as an ordinary cooked rice. As indicated, G2 has an extremely low swelling power that was well-reflected in the texture of its cooked grains that were very hard and had practically no adhesiveness (*1*). These indicate that most of the endosperm cell components, mostly starch (amylose + amylopectin) and other minor constituents, are retained within the individual grains, and only a small portion of them may have been leached out of the grain during cooking, causing poor gelatinization. It is well-known that starch granules swell to several times their initial size as a result of the absorption of water, which would cause the leaching of amylose out of the granule followed by the disruption of a crystalline order of the starch granule (*17*, *21*, *22*). The swelling of starch is primarily the property of amylopectin content, and amylose plays as both a diluent and an inhibitor of swelling, especially in the presence of lipids (*17, 23*). A low swelling power of G2 suggests that the amount of water entering into its granules is not enough to cause the leaching of a proper amount of amylose and subsequent disruption of crystallites, which are made primarily of amylopectin molecules (*17, 22*−*24*), thereby allowing the amylopectin to swell. It was reported in our preceding paper (*2*) that the preparation of starch isolation and purification from IP, as in the cases of other ordinary rice, regardless of hard or soft cooking rice (*18*, *19*), consisted entirely of well-separated individual starch granules, whereas that from G2 consisted mostly of large and intact compound starch granules in which individual granules were tightly packed. In addition, each of these compound starch granules was enclosed within a sac-like structure tolerant of mechanical as well as chemical treatments involved in starch isolation processes (*2*). It was suggested, therefore, that the low swelling power and hard cooking properties of G2 were related with the sac-like structure that would prevent or limit the entrance of a proper amount of water into the starch granules for swelling and gelatinization. One of the objectives of the present study was to elucidate the nature of the sac by studying the ultrastructure of cooked and malt-treated grains of G2, which had retained a substantial degree of hardness (**Table 1**), with the expectation that the sac could be displayed as a walled barrier of some sort around the granules. However, no such structure was evident. Instead, the bundles of Mfs embedded in the coarse and grainy starch matrix were consistently found, indicating that the sac is not an ultrastructurally identifiable independent entity but is a product formed by the interaction between molecular components of starch (amylose and amylopectin) and other basic components present. It should be recalled here that G2 contains an unusually high amount of lipids, some of which are known to occur as starch-associated lipids (starch-lipids) in milled rices (*17*), comprising 1.89%, more than four times of that (0.44%) in IP (*1*). Most milled rice is known to be comprised of typically 0.5−1.0% of starch lipids (*25*, *26*).

The lipid portion of starch-lipids in cereal starches has been demonstrated to be complexed primarily with amylose rather than amylopectin, and amylose and lipid contents were directly correlated (*27*−*29*). It is suggested, therefore, that a large portion of amylose molecules in G2 is lipid-complexed forms of amylose that would inhibit the swelling of its granules and thereby prevent the leaching of amylose from them, causing poor gelatinization during cooking. The sac-like structure surrounding the starch granules observed in the row grains of G2 endosperm by SEM (*2*), therefore, may represent a layer where a large amount of lipid-complexed amylose is accumulated around the periphery of the granules, associated with poorly swollen and, therefore, incompletely gelatinized amylopectin that is retained in the granules. When such granules are cooked, lipid-complexed amylose molecules of some types, depending on their amylose chain lengths and lipid components (*31*, *32*), may form structural features such as Mfs, and some other types may contribute to the coarseness and/or graininess of the starch matrix as shown in **Figures 2**, **3**, and **4B**.

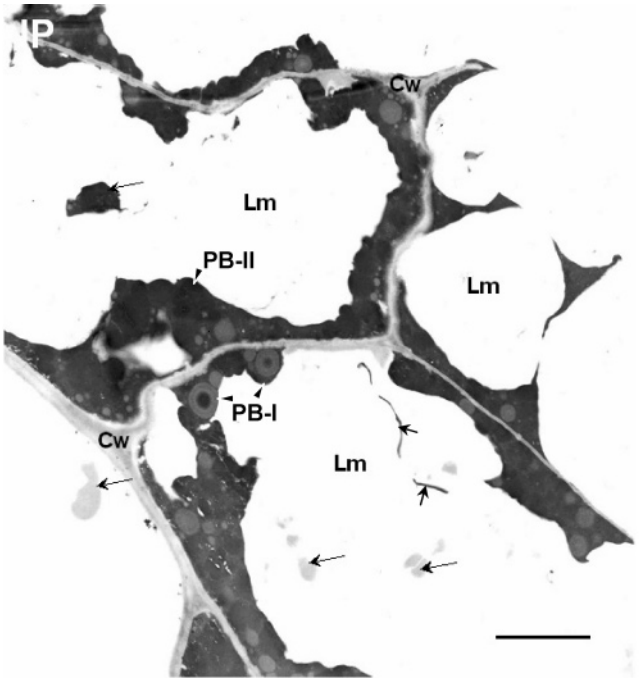

Figure 5. Low magnification view of a malt-treated cooked rice grain from IP (scale bar = 5 μm). Amorphous smooth and glossy sheetlike starch matrix and globules shown in **Figure 1** are replaced by a large empty lumen (Lm), indicating that most starch was hydrolyzed by the malt enzymes. Moderately electron-dense structures of unorganized shapes and sizes (unlabeled arrows) in the lumen are also shown. Cw = cell wall.

Malted Rice Ultrastructure. Like in the case of cooked rice grains, striking differences between IP and G2 were also clearly demonstrated when cooked rice was treated with the malt that contained amylolytic enzymes.

IP. Amorphous smooth sheets and/or globules of starch matrices occurring in cooked rice grains were no longer present due apparently to the digestion by the amylolytic enzymes present in the malt (**Figure 5**). Each endosperm cell, therefore, appeared as a large empty lumen surrounded by the cell wall and associated protein bodies (PB I and PB II) and other cytoplasmic remnants unaffected by the malt enzymes (**Figure 5**). It was not uncommon, however, to observe moderately electron-dense structures of unorganized shapes and sizes scattered randomly in the lumen (**Figure 5**). They may represent the cytoplasmic remnants of proteinaceous material remained in mature grains accumulated near the cell wall and also between amyloplasts (compound starch granules), which were not affected by the malt treatment. Protein bodies of both PB I and PB II retained their structural identity (**Figures 5** and **9A**,**B**). In fact, PB I exhibited structural details that were very similar to those occurring in raw grains (*2*). In addition, PB I in malt-treated IP rice was much clearer in appearance than thhatose in unmalted cooked rice, exhibiting characteristic internal structures (compare **Figure 9A** and **B**). This indicates that the malt treatment has a bleaching effect of removing some starch

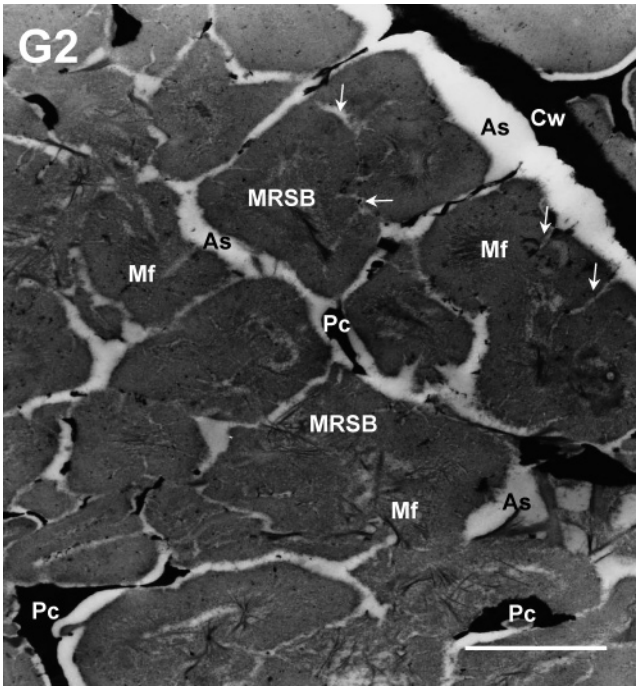

Figure 6. Low magnification view (scale bar = 5 μm) of a malt-treated endosperm cell near the central region of the G2 grain showing orderly packed malt-resistant starch bodies (MRSB). Each MRSB consisting of a coarse and grainy starch matrix in which fibrillar microfilaments (Mf) are embedded is surrounded by electron-lucent air space (As). Protein bodies and associated cytoplasmic remnants (Pc), appearing as extremely electron-dense strips, occur between MRSBs enhancing their individual profiles. Lightly stained linear gaps (unlabeled arrows) running in different directions within each MRSB are also discernible.

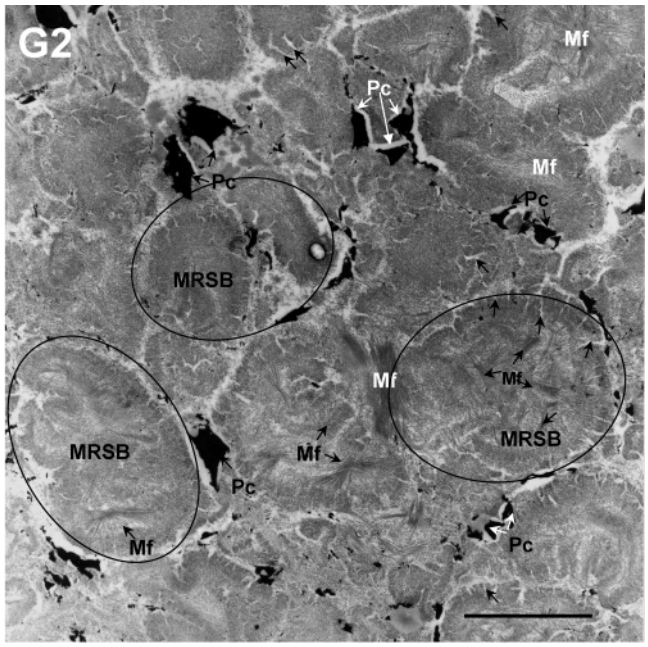

Figure 7. MRSBs in an endosperm cell near the surface of malt-treated grain from G2 showing somewhat disrupted morphology (scale bar = 5 μm). The periphery of each MRSB is serrated with radially oriented clefts (unlabeled arrows) exhibiting a saw blade appearance. Microfilaments (Mf) usually occur in the central area of each MRSB. Pc = protein bodies associated cytoplasmic remnants.

material that interferes the structural distinctiveness of PB I. PB IIs were, however, much more densely stained than those

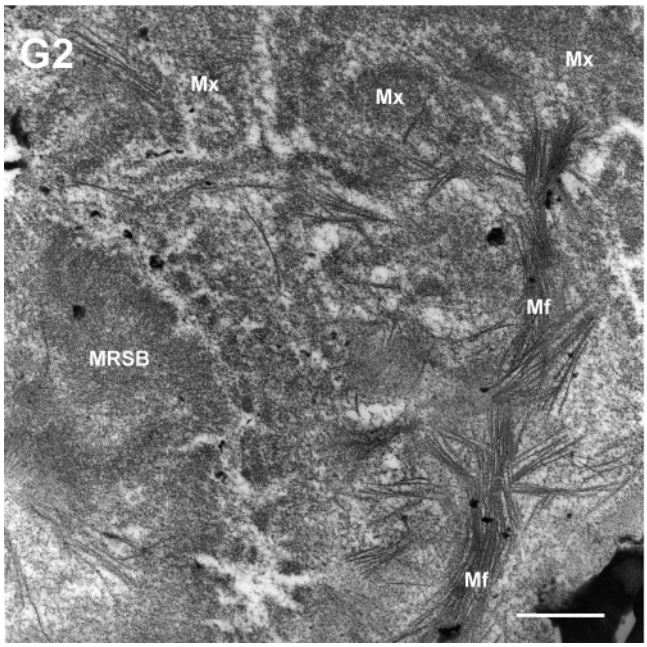

Figure 8. Higher magnification view of disrupted MRSBs in an endosperm cell near the surface of a grain from G2 showing structural details of the coarse and grainy starch matrix (Mx) and the bundles of fibrillar microfilaments (Mf) (scale bar = 1 μm). The filaments are randomly distributed throughout the matrices of disrupted MRSBs.

of PB I, causing difficulty in revealing their structural details (**Figure 9B**).

G2. In contrast to IP, the coarse and grainy starch sheets, with the bundles of Mfs present in unmalted cooked grains of G2, were not digested by the malt enzymes but remained as distinct structural features (compare **Figures 6**−**8** with **Figures 2** and **3**). In endosperm cells near the central region of the grains, they occurred as large voluminous bodies of somewhat similar sizes and shapes separated by electron-lucent spaces that helped identify the profile of individual bodies (**Figure 6**). These bodies, which may be termed as malt-resistant starch bodies (MRSBs), were similar in size and general configuration to the compound starch granules observed in thin sections of endosperm cells in situ by TEM and also to those in the isolated and fractured preparations examined by SEM (*2*). They are, therefore, believed to be the compound starch granules remaining in the grains after the malt treatment. In fact, the lightly stained linear gaps running several different directions shown clearly within each of the MRSBs (**Figure 6**) suggest that they represent the traces of boundaries of individual starch granules clustered in it. The presence of the spaces between MRSBs and the linear gaps within each of them, which were absent in unmalted cooked rice grains, reinforced the profiles of MRSBs and individual starch granules within them (compare **Figures 2** and **6**). It is suggested that the spaces and gaps arose by digestion of the starch material present in those places, which were produced by the leaching of some of the starch materials out of the granules in the process of gelatinization during cooking. The storage protein bodies and associated cytoplasmic remains, occurring near the cell wall and around the starch-packed amyloplasts (compound starch granules) in the cytoplasm of endosperm cells in raw grains (*2*), appeared in malt-treated cells as electron-dense stripes of various shapes occurring near the cell wall and also running between and around the MRSBs (**Figures 6** and **7**). These suggest further that MRSBs are indeed compound starch granules.

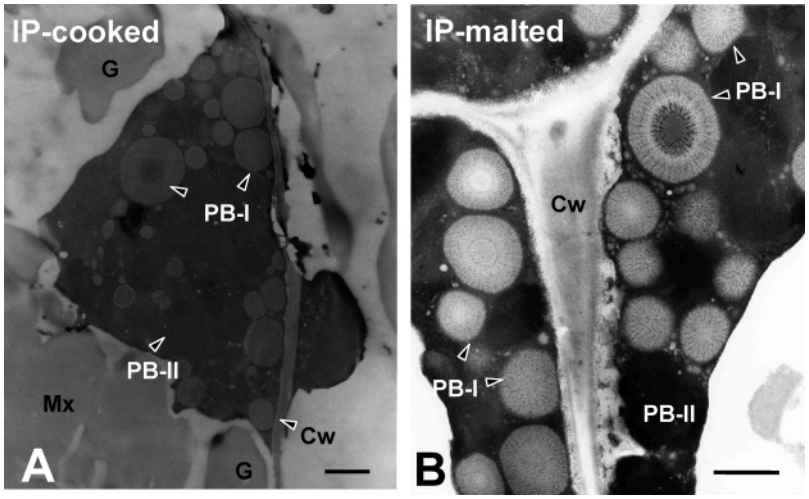

Figure 9. Higher magnification view of protein bodies and associated cytoplasmic remnants retained in cooked (**A**) and malt-treated (**B**) rice of IP. (**A**) From cooked IP (scale bar = 1 μm). Spherically shaped prolamin protein bodies (PB 1) of various sizes are clearly distinguishable from other cytoplasmic remnants nearby. Irregularly shaped glutelin protein bodies (PB II) appear extremely electron-dense and merged with other remnants, showing no clear morphological profiles. Cw = cell wall; G = starch globules; and Mx = starch matrix. (**B**) From malt-treated IP (scale bar = 1 μm). Structural details of spherically shaped prolamin protein bodies (PB I) are much clearer than those in unmalted cooked rice shown in **panel A**. Structural details of irregularly shaped glutalin protein bodies (PB II) are, however, not clearly discernible. Cw = cell wall.

The MRSBs in endosperm cells near the peripheral region of the grains (**Figures 7** and **8**) were not as well-organized as those present near the central region shown in **Figure 6**. Some of the relatively well-defined individual MRSBs in these cells (**Figures 7** and **8**) were surrounded by a disorganized starch matrix produced apparently by the dispersion or disruption of nearby MRSBs, caused apparently by the action of malt enzymes. The coarseness or graininess of the starch matrix and the Mfs were, however, structurally intact in these disrupted MRSBs (**Figures 7** and **8**). In fact, these structures were somewhat reinforced in appearance. This suggests that some starch material in the matrix is digested by the malt enzymes, thereby unmasking their (matrix and filaments) structural existence more sharply (compare **Figure 3** with **Figure 8**), as in the cases of MRSBs by the electron-lucent spaces between them and of individual starch granules within each MRSB by the linear gaps. In addition, the peripheries of most of these MRSBs were serrated with radially oriented clefts exhibiting a sow blade appearance (**Figure 7**). The clefts apparently represent the eroded areas caused by the action of malt enzymes.

The digestion of cereal starch by amylolytic enzymes has been the subject of many investigations in recent years due to its analytical and possible nutritional significance in the dietary fiber concept, and the starch that is resistant to amylolytic enzymes has been called resistant starch (RS) (*32*−*36*). The present study reported in this paper demonstrates clearly that the IP starch, a low-amylose rice that has a high quality of cooked rice, is highly susceptible to the malt enzymes, whereas the G2 starch, a high-amylose rice that has an unacceptable quality of cooked rice, is extremely resistant to the enzymes. Starches, including those of rice, with high contents of amylase and lipid, like G2, have been well-demonstrated to be highly resistant to anylolytic enzymes (*32*−*35*). It is believed, therefore, that MRSBs consisting of the coarse matrix with Mfs embedded in it, as discussed earlier on the nature of the sac-like structure of the compound starch granules (*2*), represent a mixture of amylase−lipid complexes with different rates and extents of resistance to the malt enzymes, plus poorly swollen and, therefore, incompletely gelatinized amylopectin that was retained in the granules of whole grains.

The Mfs, one of the most striking features and unique to cooked rice of G2, which are extremely resistant to amylolytic enzymes and are structurally rigid, may show some ultrastructural similarity to the microfibrils of the plant cell wall. However, it is very unlikely that these two are compositionally related to each other since the former is formed in the starch granules in which no enzymes involved in the cell wall synthesis are present (*17*). It is well-known that the synthetic pathways inolved in cell wall synthesis, from the formation of cellulose (unbranched straight chains of glucose residues by β-1,4 linkages) to microfibrils, fibrils, and finally to the deposition of these cell wall precursor material to the cell wall, are taking place in the cytoplasm through the endomembrane system including a Golgi apparatus (*37*). Of the higher contents of dietary fibers in G2, which were more than 2 times those in IP (*1*), cellulose, hemicellulose, or other cell wall components are, therefore, unlikely involved in the formation of G2 Mfs. However, it is possible that the G2 Mfs may behave as some of the components of dietary fiber, like cellulose or hemicellulose, during the assaying processes and participated in the content values given (*1*). In fact, it has been widely recognized that the presence of RS, which has physiological effects similar to some of the components of dietary fibers, has important implications for dietary fiber determination (*34*, *35*, *38*, *39*). The cell wall components such as cellulose, hemicellulose, and their associated structures, which are highly indigestible in alimentary canal of mammals including humans, have been considered traditionally to constitute the major part of dietary fibers (*40*−*42*).

It should be noted that the ultrastructurally identifiable filamentous structures, like the Mfs of G2 that are similar in appearance to the microfibrils of the cell wall (*43*), can be formed during food processing from the storage polysaccharides, such as starch, which is made of α-linkages of glucose residues. Cellulose, the major structural polymer of plants made of long and straight chains of β-linkages of glucose units, is the most commonly known form of polysaccharide to form structurally rigid filamentous structures such as microfibrils and fibrils of the cell wall (*37*, *43*).

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LITERATURE CITED

(1) Kang, H. J.; Hwang, I. K.; Kim, K. S.; Choi, H. C. Comparative structure and physicochemical properties of Ilpumbyeo, a high-quality japonica rice, and its mutant, Suweon 464. *J. Agric. Food Chem.* **2003**, *51*, 6598−6603.

(2) Kim, K. S.; Kang, H. J.; Hwang, I. K.; Hwang, H. G.; Kim, T. Y.; Choi, H. C. Comparative ultrastructure of Ilpumbyeo, a high-quality of japonica rice, and its mutant Suweon 464: Scanning and transmission electron microscopy studies. *J. Agric. Food Chem*. **2004**, *52*, 3876−3883.

(3) Yun, J. Y.; Choi, K. G.; Kim, J. K. Effect of anaerobic treatment on carbohydrate-hydrolytic enzyme activities and free amino acid contents in barley malt. *J. Crop Sci*. **1998**, *43*, 19−22.

(4) Suh, H. J.; Chung, S. H.; Whang, J. H. Characteristics of Shickhae produced with malt of naked barley, covered barley, and wheat. *Korean J. Food Sci. Technol.* **1997**, *29*, 716−721.

(5) Karnovsky, M. J. A formalehyde−glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol*. **1965**, *27*, 137A.

(6) Spurr, A. R. A low viscosity epoxy resin-embedding medium for electron microscopy. *J. Ultrastruct. Res*. **1969**, *26*, 31−43.

(7) Bechtel, D. B.; Pomeranz, Y. The rice kernel. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemist: St. Paul, MN, 1980; pp 73−113.

(8) Bechtel, D. B.; Juliano, B. O. Formation of protein bodies in the starch endosperm of rice (*Oryza sativa* L.): A reinvestigation. *Ann. Bot*. **1980**, *45*, 503−509.

(9) Tanaka, K.; Sugimoto, T.; Ogawa, M.; Kasai. Z. Isolation and characterization of two types of protein bodies in the rice endosperm. *Agric. Biol. Chem*. **1980**, *44*, 1633−1639.

(10) Tanaka, Y.; Hayashida, S.; Hongo, M. The relationship of the feces protein particles to rice protein bodies. *Agric. Biol. Chem*. **1975**, *39*, 515−518.

(11) Tanaka, Y.; Resurreccion, A. P.; Juliano, B. O.; Bechtel, D. B. Properties of whole and undigested fraction of protein bodies of milled rice. *Agric. Biol. Chem*. **1978**, *42*, 2015−2023.

(12) Baber, D. L.; Lott, J. N. A.; Yang, H. Properties of rice (*Oryza sativa* L.) fecal protein particles: light and electron microscopic observations. *J. Cereal Sci*. **1998**, *27*, 83−93.

(13) Sivak, M. N.; Preiss, J. Starch: basic science to biotechnology. In *Advances in Food and Nutrition Research*; Sivak, M. N., Preiss, J., Eds.; Academic Press: San Diego, 1998; Vol. 18, pp 163−170.

(14) Juliano, B. O. Polysaccharides, proteins, and lipids of rice. In *Rice Chemistry and Technology*; Juliano, B. O., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1985; pp 59−174.

(15) Mashall, W. E. Starch gelatinization in brown and milled rice: a study using differential scanning colorimetry. In *Rice Science and Technology*; Marshall, W. E., Wadworth, J. I., Eds.; Marcel Dekker: New York, 1994; pp 205−227.

(16) Chrastil, J. Effect of storage on the physicochemical properties and quality factors of rice. In *Rice Science and Technology*; Marshall, W. E., Wadworth, J. I., Eds.; Marcel Dekker: New York, 1994; pp 49−81.

(17) Zhau, Z.; Roberds, K.; Helliwell, S.; Blanchard, C. Composition and functional properties of rice. *Int. J. Food Sci. Technol*. **2002**, *37*, 849−868.

(18) Ong, M. H.; Blanshard, J. M. V. Texture determinations of cooked, parboiled rice. I. Rice starch amylose and fine structure of amylopectin. *J. Cereal Sci*. **1995**, *21*, 251−260.

(19) Ong, M. H.; Blanshard, J. M. V. Texture determinations of cooked, parboiled rice. II. Physicochemical properties and leaching behavior of rice. *J. Cereal Sci*. **1995**, *21*, 261−269.

(20) Juliano, B. O. An international survey of methods used for the cooking and eating qualities of milled rice. In *IRRI Research Paper Series*; IRRI: Los Banos, The Philippines, 1982; No. 77.

(21) Blanshard, J. M. V. Starch granule structure and function: a physicochemical approach. In *Starch properties and Potential*; Galliard, T. Ed.; Wiley Press: Chichester, UK, 1987; pp 16−54.

(22) Lii, C. Y.; Tsai, M. L.; Tseng, K. H. Effect of amylose content on the rheological property of rice starch. *Cereal Chem*. **1996**, *73*, 415−420.

(23) Tester, R. F.; Morrison, W. R. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipid. *Cereal Chem*. **1990**, *67*, 551−557.

(24) Tester, R. F.; Morrison, W. R. Swelling and gelatinization of cereal starches. II. Waxy rice starches. *Cereal Chem*. **1990**, *67*, 558−563.

(25) Choudhury, N. H.; Juliano, B. O. Effects of amylose content on the lipids of mature rice grain. *Phytochemistry* **1980**, *19*, 1385−1389.

(26) Kawashima, K.; Kiribuchi, T. Studies on lipid components and heat dependent pasting behavior of nonwaxy and waxy rice starches. *Kaseigaku Zasshi* **1980**, *31*, 625−628.

(27) Tester, F. F.; Morrison, W. Swelling and gelatinization of cereal starches. III. Some properties of waxy and normal nonwaxy barley starches. *Cereal Chem*. **1992**, *69*, 654−658.

(28) Morrison, W. R.; Tester, F. R.; Snape, C. E.; Law, R. V.; Gidley, M. J. Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. *Cereal Chem*. **1993**, *70*, 385−391.

(29) Morrison, W. R.; Law, R. V.; Snape, C. E. Evidence for inclusion complexes of lipids with α-amylose in maize, rice, and oat starches. *J. Cereal Sci*. **1993**, *18*, 107−109.

(30) Biliaderis, C. D.; Galloway, G. I. Gelatinization behavior of amylose-v complexes; structure−property relationship. *Carbohydr. Res*. **1989**, *189*, 31−49.

(31) Galloway, G. I.; Biliaderis, C. G.; Stanley, D. W. Properties and structure of amylose-glyceryl monostearate complexes formed in solution of an extrusion of wheat flour. *J. Food Sci.* **1989**, *54*, 950−957.

(32) Seneviratne, H. D.; Biliaderis, C. G. Action of α-amylases on amylose-lipid complex superstructures. *J. Cereal Chem*. **1991**, *13*, 129−143.

(33) Kitahara, K.; Tanaka, T.; Suganuma, T.; Nagahama, T. Release of bound lipids in cereal starches upon hydrolysis by glucoamylase. *Cereal Chem*. **1997**, *74*, 1−6.

(34) Berry, C. S. Resistant starch: formation and measurement of starch that survives exhausative digestion with amylolytic enzymes during the determination of dietary fiber. *J. Cereal Sci*. **1986**, *4*, 301−314.

(35) Ring, S. G.; Gee, J. M.; Whitham, P. O.; Johnson, I. T. Resistant starch: its chemical form in foodstuffs and effect on digestibility in vitro. *Food Chem*. **1988**, *28*, 97−109.

(36) Russell, P. L.; Berry, C. S.; Grenwell, P. J. Characterization of resistant starch from wheat and maize. *J. Cereal Sci.* **1989**, *9*, 1−15.

(37) Smith, C. J. Carbohydrate biochemistry. In *Plant Biochemistry and Molecular Biology*; Lee, P. J., Leegood, R. C., Eds.; John Wiley & Sons: New York, 1999; pp 81−118.

(38) Asp, N. G.; Johansson, C. G.; Hallmer, H.; Siljestrom, M. Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem*. **1983**, *31*, 476−482.

(39) Englyst, H. N.; Cummings, J. H. Resistant starch, a new food component: a classification of starch for nutritional purposes. In *Cereals in a European Context*. *First European Conference on Food Science and Technology*; Morton, I. D., Ed.; Ellis Horwood Ltd.: Chichester, UK, 1987; pp 221−233.

(40) Hipsley, E. H. Dietary fiber and pregnancy toxemia. *Br. Med. J.* **1953**, *2*, 420.

(41) Trowell, H. Definition of dietary fiber and hypothesis that is a protective factor in certain disease. *J. Am. Clin. Nutr*. **1976**, *29*, 417−425.

(42) Palacio, J. C.; Rembeau, J. L. Dietary fiber: a brief review and potential application to enternal nutrition. *Nutr. Clin. Practice* **1990**, *5*, 99−100.

(43) Gunning, B. E. S.; Steer, M. W. Plasma membrane, microfibrils in the cell wall. In *Ultrastructure and Biology of Plant Cells*; Crane Russak and Co., Inc.: New York, 1975; pp 186−191.

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