JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Milling of Rice Grains. The Degradation on Three Structural Levels of Starch in Rice Flour Can Be Independently Controlled during Grinding

Thuy T. B. Tran, Kinnari J. Shelat, Daniel Tang, Enpeng Li, Robert G. Gilbert, and Jovin Hasjim*

The University of Queensland, Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD 4072, Australia

Supporting Information

ABSTRACT: Whole polished rice grains were ground using cryogenic and hammer milling to understand the mechanisms of degradation of starch granule structure, whole (branched) molecular structure, and individual branches of the molecules during particle size reduction (grinding). Hammer milling caused greater degradation to starch granules than cryogenic milling when the grains were ground to a similar volume-median diameter. Molecular degradation of starch was not evident in the cryogenically milled flours, but it was observed in the hammer-milled flours with preferential cleavage of longer (amylose) branches. This can be attributed to the increased grain brittleness and fracturability at cryogenic temperatures, reducing the mechanical energy required to diminish the grain size and thus reducing the probability of chain scission. The results indicate, for the first time, that branching, whole molecule, and granule structures of starch can be independently altered by varying grinding conditions, such as grinding force and temperature.

KEYWORDS: rice, grains, cryogenic milling, hammer milling, particle size, starch damage

INTRODUCTION

Starch is the major component in most cereal grains. The structure of starch in cereal grains can be simply grouped into six levels (Figure 1): individual linear branches of starch molecules (level 1) where anhydroglucose units are linked together by α-1,4 glycosidic bonds, macromolecular branched structure (level 2) where the linear glucan branches are joined together by α-1,6 glycosidic bonds to form amylopectin and amylose (levels 1 and 2 are "molecular"), semicrystalline structure (level 3) formed by double helices of amylopectin branches, growth ring structure (level 4) formed by several alternating crystalline and amorphous lamellae, granular structure (level 5) consisting of several growth rings, and whole grain structure (level 6) where starch granules interact with protein, lipid, nonstarch polysaccharides, and other components in the grains. Other levels of starch structures, such as superhelical[1] and blocklet structures,[2] are excluded here as they are not commonly studied.

Damage to starch granules (usually termed "starch damage"), the disruption of level 5 starch structure, commonly occurs during grinding (milling) of cereal grains (level 6 starch structure). Examples of the damage are broken starch granules with exposed interior and granule fragments.[3,4] Different milling or grinding processes have been shown to produce different degrees of damage to starch granules in flour depending on the mechanical forces and temperature during the grinding process.[5−7]

The presence of damaged starch granules improves the water-absorption capacity of flour.[6,8] This property is important to increase the amount of water absorbed by the flour dough, required to achieve high loaf-volume of bread[9] and desirable texture for noodles.[6] Damaged starch granules are also more susceptible to enzyme hydrolysis than the intact starch granule counterparts.[3,4] Hence, during yeast fermentation of bread dough, damaged starch granules can be easily hydrolyzed by yeast α-amylase to produce maltose, which is consequently utilized as the substrate for fermentation.[9]

Although a low degree of damage to starch granules can facilitate water absorption and improve starch swelling, excessively damaged starch granules undergo less swelling during cooking and have higher solubility in cold water than the intact starch granule counterparts. This is attributed to the degradation of starch molecules (structural levels 1 and 2), mainly amylopectin, and starch crystalline structure (level 3 structure) that occurs during grinding, producing highly soluble, low molecular-weight molecules.[3,10−16] The presence of this low molecular weight amylopectin increases the cooking loss and reduces the water uptake during cooking, such as seen in noodles.[6] Furthermore, excess damaged starch granules in flour can result in a lower loaf-volume bread because of rapid hydrolysis of starch by α-amylase during fermentation, reducing the water-holding capacity of the dough and releasing the absorbed water.[8] Therefore, controlled grinding processes that reduce the particle size of grains (level 6 structure) and increase the damage to starch granules (level 5 structure) with limited degradation on the starch molecular structure (level 1 and 2 structures) will benefit the cereal industry, as they can improve the water absorption of the flour without sacrificing the functional properties of the starch.

In addition to the preceding considerations, milling or grinding is required to disrupt protein and cell-wall matrices, facilitating the release of starch granules from the grains for subsequent starch structure characterization.[17] For this purpose, it is important that the milling or grinding processes do not cause

Received: December 31, 2010
Accepted: March 8, 2011
Revised: March 4, 2011
Published: March 08, 2011

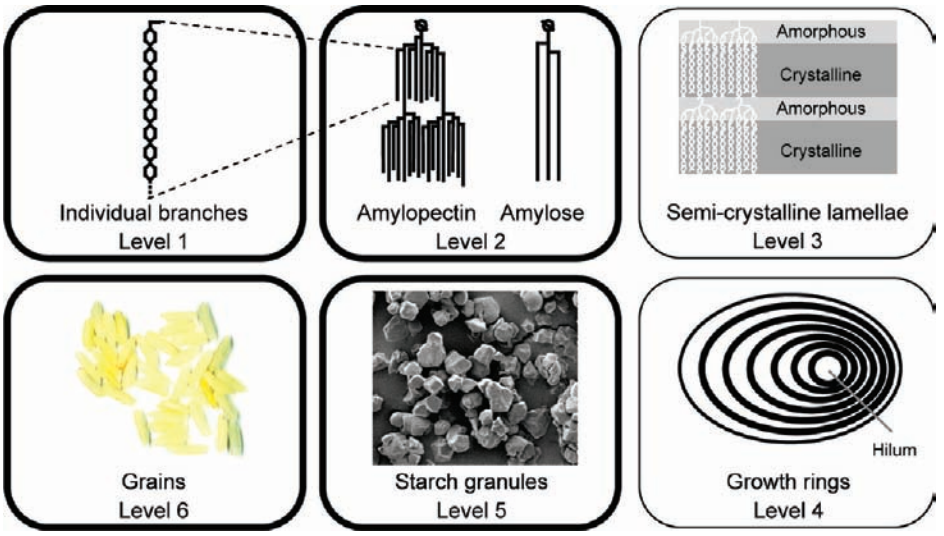

Figure 1. Six levels of starch structures. Bold borders show the levels of the structures that are discussed in the text.

degradation of starch molecules (level 1 and 2 structures) in order to obtain an accurate characterization of the starch molecular structure. For example,[18] laboratory-scale wet-milling, commonly used to isolate starch granules from grains with minimal damage,[4] results in amylopectin with smaller average hydrodynamic size than that obtained without wet-milling. It is likely that the endogenous enzymes in the grains become active during soaking of grains in sodium bisulfite solution to weaken the protein matrix in the grains and/or in sodium chloride solution for protein removal. Other less likely possibilities include selective loss of starch with larger amylopectin during wet milling and mechanical scission of the bonds in larger molecules. Mild cryogenic milling is a better alternative process to wet milling as it conserves the molecular structure of starch;[18] however, cryogenic milling has been reported to damage starch granular structure (level 5 structure), although the level of damage is less than most grinding processes.[3,5] Thus, for starch structure characterization, it is necessary to optimize the cryogenic-milling procedure to completely extract starch from grains without molecular degradation.

The objective of this study is to understand the relationship between the damage to starch granules (level 5 structure) and the degradation of the starch molecular structure (level 2 structure) and the branching structure within the starch molecules (level 1 structure) during particle size reduction (grinding) of rice grains (level 6 structure). Rice (*Oryza sativa* L.) grains are used in this study as rice is one of the most widely grown crops for food. Although the grains are mostly consumed as cooked whole grains, rice flour is commonly produced via grinding or milling of broken rice grains and used in baby foods, noodles, puddings, and many Asian cuisines. Since rice is hypoallergenic, colorless, and relatively bland tasting, the flour has been used as a substitute for wheat flour for celiac patients, for example, in making gluten-free bread.[7,19]

In this study, rice grains were dry-ground using cryogenic and hammer milling for comparison. The use of cryogenic milling on cereal grains is still limited and its effects on the grain structure, especially on the starch structure in the grain, have not been well studied. Cryogenic grinding has been applied to grind spice seeds for better quality.[20,21] At cryogenic temperatures, the seeds become brittle, allowing them to crumble easily to a fine and consistent size. Cryogenic temperatures also lower the mechanical energy required to reduce the particle size of seeds.[20−22] Hence, grinding of rice grains at cryogenic temperature has the potential to prevent the mechanical degradation of starch molecular structure (level 1 and 2 structures) during grinding. This is the first study to utilize cryogenic-milling process at different grinding times, in addition to the common hammer-milling process at ambient temperature with screens of different opening sizes, to produce a series of rice flours with various particle sizes (level 6 structure) and degrees of damage to the starch granules (level 5 structure) in order to draw a mechanistic understanding of the degradation of starch molecular structure (level 1 and 2 structures) during grinding.

Table 1. Cryogenic Milling Conditions for Grinding Rice Grains

sample code	grinding time/cycle (min)	no. of cycles	total grinding time (min)
CM5C1	5	1	5
CM5C2	5	2	10
CM10C2	10	2	20
CM10C3	10	3	30
CM10C4	10	4	40

MATERIALS AND METHODS

Polished long-grain rice grains were purchased from a local grocery store. The chemical composition of the rice grains (on the basis of wet-grain weight) as provided by the manufacturer is as follows: 79% carbohydrates, 7% protein, and 1% lipid. Protease from *Streptomyces griseus* (type XIV) and lithium bromide (ReagentPlus) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Dimethyl sulfoxide (DMSO, GR for analysis ACS) was purchased from Merck & Co, Inc. (Kilsyth, VIC, Australia). Total starch (AA/AMG) assay kit, starch damage assay kit, and isoamylase from *Pseudomonas* sp. were purchased from Megazyme International Ltd. (Co. Wicklow, Ireland).

Cryogenic Milling of Rice Grains. Rice grains were ground using a cryogenic mill (freezer/mill 6870 SPEX, Metuchen, NJ, USA). The grains were prefrozen in liquid nitrogen bath (−210 to −196 °C) for 5 min and then pulverized with a magnetically driven impactor at 10 s^{-1} in

Table 2. Hammer Milling Conditions for Grinding Rice Grains

sample code	mill screen opening size (μm)	no. of passes
HM1500P1	1500	1
HM1000P1	1000	1
HM500P1	500	1
HM500P2	500	2
HM500P3	500	3

the liquid nitrogen bath. The grains were subjected to 1−2 cycles of 5 min grinding or 2−4 cycles of 10 min grinding. Each cycle of grinding included a 2 min refreezing period after a period of grinding. The refreezing step was to ensure the rice grains were at cryogenic temperature, as the grain temperature may rise during grinding. The total cryogenic milling times of the rice grains were 5, 10, 20, 30, and 40 min, as summarized in Table 1.

Hammer Milling of Rice Grains. Rice grains were ground by passing through a hammer mill (Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) at ambient temperature. The opening sizes of the hammer-mill screens used were 500, 1000, and 1500 μm. In addition, rice grains were ground through the hammer mill with the 500 μm screen for two and three passes to produce flour samples with greater damage to the starch granules. The hammer mill was cleaned in between passes to avoid cross contamination of the flour samples. The hammer-milling conditions for grinding the rice grains are summarized in Table 2. The temperature of the rice flour immediately after passing through the hammer mill with 500 μm screen was about 40−45 °C.

Scanning Electron Microscopy. The structure of rice flour particles (level 6 starch structure) coated with platinum to a thickness of ~20 nm was analyzed using a scanning electron microscope (SEM, JSM-6610, JEOL, Tokyo, Japan) at the Centre for Microscopy and Microanalysis, the University of Queensland. The SEM was operated at 3.0 kV, spot size of 45, and working distance of 12 mm. Magnifications at 100× and 1000× were used to observe the particle size from a large population of flour particles and the surface morphology of the small flour particles, respectively.

Particle Size of Rice Flour. The particle size of rice flour was measured using a Mastersizer 2000 with Hydro MU (Malvern Instruments Ltd., Malvern, U.K.) following the method of Mahasukhonthachat et al.[23] Rice flour (about 250 mg) was dispersed in 5 mL of distilled water at least 30 min before the measurement to minimize the differences caused by cold-water swelling of flour particles. The dispersed flour was added to circulating water until obscuration >10% was recorded. The diameters at 10th percentile, $D(v,0.1)$, 50th percentile or volume-median, $D(v,0.5)$, and 90th percentile, $D(v,0.9)$, were determined in duplicate (and sometimes more) using the general purpose model at 2,000 rpm.

Starch Granule Damage. The degree of damage to starch granules (level 5 structure) in rice flour sample was determined based on the susceptibility to amylolytic enzyme hydrolysis, using Megazyme starch damage assay kit in triplicate, following the procedure provided by the manufacturer.[24] The amount of starch in rice flour was determined in triplicate using Megazyme total starch assay kit following the procedure provided by the manufacturer[25] after the rice flour (100 mg) was wetted with 80% v/v ethanol (0.2 mL) and then dissolved in DMSO (2 mL) in a boiling water bath for 5 min. The starch content was used in calculating the damage to starch granules. The moisture content of rice flour was determined from the difference in the flour weight before and after drying in an oven at 110 °C for 24 h following the method of Hasjim et al.[26] The molar mass conversion ratio from glucose to anhydroglucose (the starch monomer unit) is 0.9. The degree of damage to starch granules was calculated as follows:

$$\%\text{starch granule damage} = \frac{\text{wt of glucose from damaged starch granules} \times 0.9}{\text{dry wt of starch in flour}} \times 100\%$$

where the weight of glucose from damaged starch granules was obtained from the rapid (10 min) starch hydrolysis using Megazyme starch damage assay kit, and the dry weight of starch in flour was obtained from the complete starch hydrolysis using Megazyme total starch assay kit adjusted to the solid content of the flour.

Molecular Size Distribution of Fully Branched and Debranched Starch. For molecular size distribution of (whole) fully branched starch (level 2 structure), starch was extracted from rice flour (2 mg) and dissolved in DMSO solution containing lithium bromide (0.5% w/w) (DMSO/LiBr) following the method of Syahariza et al.[18] with a minor modification as follows. The extraction/dissolution method involves protease treatment to increase the solubility of the protein in the flour, dissolution in DMSO/LiBr solution to remove insoluble nonstarch materials, and, finally, ethanol precipitation to separate starch from soluble nonstarch components. In addition to those treatments, rice flour was treated with sodium bisulfite solution (0.45% w/w sodium metabisulfite in distilled water, 0.25 mL/mg flour) at 37 °C for 15 min after the protease treatment, followed by centrifugation at 4000g for 10 min to remove the supernatant before the dissolution of the treated flour in DMSO/LiBr solution. The additional step of sodium bisulfite treatment was included to break the disulfide bonds of protein in rice flour.

For molecular size distribution of individual branches of starch molecules (debranched starch, level 1 structure), starch was extracted from a larger amount of rice flour (~5 mg) using the same method as described for the sample preparation of fully branched starch (level 2 structure). The extracted starch in 1 mL of DMSO/LiBr solution was precipitated with 3 mL of absolute ethanol and then debranched using isoamylase following the method of Hasjim et al.[26]

The molecular size distributions of fully branched and debranched starches from rice flour were analyzed using a size exclusion chromatography (SEC) system (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) equipped with a refractive index detector (RID, RID-10A, Shimadzu, Kyoto, Japan) following methods given elsewhere.[27−29] The size distribution was plotted as SEC weight distribution, $w(\log V_h)$, derived from RID signals against hydrodynamic radius, R_h. The weight distributions of fully branched and debranched starches are denoted as $w_{br}(\log V_h)$ and $w_{de}(\log V_h)$, respectively. The molecular size distribution of debranched starch was also plotted as the number distribution, $N_{de}(\overline{X})$, against average degree of polymerization (DP), $\overline{X}$. For linear polymers (including debranched starch), the (debranched) number distribution, $N_{de}(X)$, can be obtained from the corresponding weight distribution, $w_{de}(\log V_h)$, as follows:[29−31]

$$w_{de}(\log V_h) = X^2 N_{de}(X)$$

The DP of linear branches was calculated from the V_h using the Mark−Houwink equation.[29,30] However, the DP obtained from the Mark−Houwink parameters is prone to error for small glucan chains, and thus is only semiquantitative for the short branches. Further information of the SEC analysis is available in the Supporting Information.

For statistical purposes, the molecular size distributions of fully branched and debranched starches (level 2 and 1 structures, respectively) were reduced to single parameters that can be correlated with the volume-median diameter of flour particles (level 6 structure) and the damage to starch granules (level 5 structure). Hence, the average hydrodynamic radius, $\overline{R}_h$, of fully branched molecules and the slope, T, of the number distribution of debranched starch, $N_{de}(\overline{X})$, were

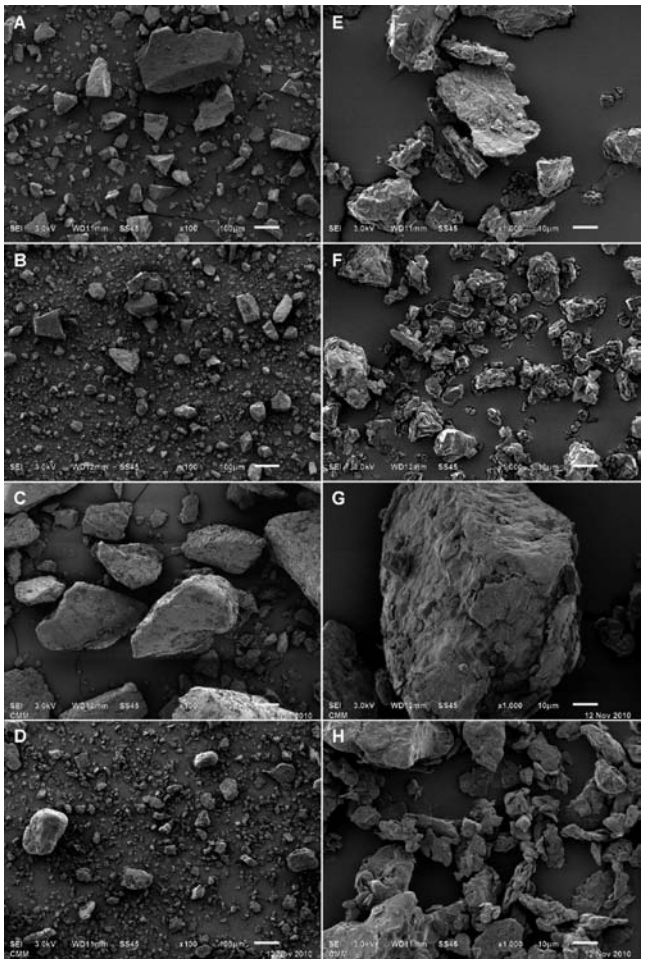

Figure 2. Scanning electron micrographs of selected rice flours. (A) CM5C1, (B) CM10C2, (C) HM1500P1, and (D) HM500P3 at 100× magnification with a bar indicating 100 μm, and (E, F, G, and H) at 1000× magnification with a bar indicating 10 μm, respectively.

determined following the method of Vilaplana and Gilbert[30] as follows:

$$\log(\bar{R}_h) = \frac{\int_{-\infty}^{\infty} w_{br}(\log V_h)\, d\log R_h}{\int_{-\infty}^{\infty} \frac{w_{br}(\log V_h)}{\log R_h} d\log R_h}$$

$$N_{de}(\bar{X}) = \bar{X}\exp(-T\bar{X} + C)$$

where C is constant.

Statistical Analysis. The mean values of the starch content of flour, the particle size of flour (level 6 structure), and the damage to starch granules (level 5 structure) were analyzed by analysis of variance (ANOVA) using Minitab 16 (Minitab Inc., State College, PA, USA). The general linear model and Tukey's pairwise comparisons with confidence level at 95.0% were used in performing the ANOVA. The correlations between molecular degradation, in terms of average hydrodynamic radius, $\bar{R}_h$, of fully branched molecules (level 2 structure) and the slope, T, of the number distribution, $N_{de}(\bar{X})$, of debranched starch (level 1 structure), with the volume-median diameter of flour particles (level 6 structure) and the damage to starch granules (level 5 structure) were also analyzed using Minitab 16.

RESULTS

Scanning Electron Microscopy. Figure 2 shows the SEM images of flour particles (level 6 starch structure) from selected rice flour samples: 1 cycle of 5 min cryogenic milling (CM5C1), 2 cycles of 10 min cryogenic milling (CM10C2), 1 pass through the hammer mill with 1500 μm screen (HM1500P1), and 3 passes through the hammer mill with 500 μm screen (HM-500P3). All cryogenically milled and hammer-milled flours had similar polygonal (and hence highly aspherical) shapes. The particle size as observed from a larger population of rice flour particles at 100× magnification was on the order of HM1500P1 > CM5C1 > CM10C2 ≈ HM500P3 (Figure 2C, A, B, and D, respectively). Furthermore, the small particles of the cryogenically milled rice flours, as observed at 1000× magnification, had jagged edges and corners (Figure 2E and F). In contrast, the small particles of the hammer-milled flours had smooth surfaces and round corners (Figure 2G and H). Isolated rice starch granules are reported to have diameters of 3 to 8 μm, which are easily observed using SEM at 1500× magnification.[32] However, starch granules in the flour particles of all samples were not easily observed at 1000× magnification although few particles with diameters smaller than those reported for rice starch granules were observed in the SEM images (Figure 2F and H), indicating that the starch granules in the rice flours were still embedded in the cell-wall matrices.

Particle Size of Flour. Although the flour particles (level 6 starch structure) of the cryogenically milled and hammer-milled rice flours were not spherical, the SEM images showed that they had similar shapes (Figure 2). Thus, the particle sizes of the cryogenically milled and hammer-milled flours can be meaningfully compared. The $D(v,0.1)$, $D(v,0.5)$, and $D(v,0.9)$ flour-particle diameter values of all rice flour samples are listed in Table 3.

It is essential to be aware that light-scattering devices, such as the Mastersizer 2000 used here, which do not involve any separation step, cannot give a true size distribution (although they are often supplied with software which purports to do this). Obtaining a size distribution without separation requires making assumptions on the nature of the distribution; such assumptions, with a few exceptions, cannot be tested with the instrumentation (see Chiou et al.[33] for an example of this). Only the appropriate mean size is generally valid. Moreover, even the mean size for aspherical particles, such as those produced here, is somewhat devoid of meaning. Nevertheless, the size information provided by the instrument can be used to analyze the trends of particle sizes among different samples with similar morphology and structure, as is the present case.

The trends of the changes in the three particle size parameters, $D(v,0.1)$, $D(v,0.5)$, and $D(v,0.9)$, after milling were similar (Table 3). For further discussion, only 50th percentile or volume-median diameter, $D(v,0.5)$, is used as the particle size parameter to investigate the effects of the milling processes on starch structure.

The particle size of the cryogenically milled flours became significantly smaller (Table 3) as the total grinding time increased up to 20 min (Table 1), which is consistent with the SEM images (Figure 2A and B). The volume-median diameter of the flour particles was reduced from 149 μm in the rice flour cryogenically milled to a total grinding time of 5 min (CM5C1) to 34 μm in the rice flour cryogenically milled to a total grinding time of 20 min (CM10C2); the volume-median diameter of the rice flour particles, however, was not significantly

Table 3. Particle Size and Degree of Damaged Starch Granules of Rice Flours after Different Grinding Treatments

	particle size[a] (μm)			
sample code	10th percentile $D(v,0.1)$	vol-median diam or 50th percentile $D(v,0.5)$	90th percentile $D(v,0.9)$	degree of damaged starch granules[a] (%)
CM5C1	17.6 ± 0.6 c	149 ± 12 d	610 ± 11 f	4.2 ± 0.1 a
CM5C2	13.9 ± 0.5 b	115 ± 6 c	480 ± 24 e	5.4 ± 0.1 b
CM10C2	7.5 ± 0.1 a	34 ± 0 ab	171 ± 1 a	11.6 ± 0.2 c
CM10C3	6.6 ± 0.2 a	32 ± 1 a	152 ± 2 a	12.0 ± 0.1 c
CM10C4	6.5 ± 0.2 a	30 ± 1 a	138 ± 3 a	14.9 ± 0.1 d
HM1500P1	75.0 ± 4.3 e	564 ± 22 f	1266 ± 23 h	4.4 ± 0.8 a
HM1000P1	71.6 ± 2.6 e	478 ± 6 e	904 ± 16 g	5.6 ± 0.1 b
HM500P1	21.0 ± 0.9 d	158 ± 3 d	437 ± 6 d	17.0 ± 0.5 e
HM500P2	12.8 ± 0.7 b	56 ± 4 b	329 ± 3 c	25.2 ± 0.2 f
HM500P3	11.4 ± 0.1 b	42 ± 2 ab	227 ± 12 b	26.1 ± 0.5 f

[a] Mean ± standard deviation from, at least, triplicate. Values with different letters are significantly different at $p < 0.05$.

reduced when the flour was further ground using cryogenic milling up to a total grinding time of 40 min (CM10C4).

The volume-median diameter of the hammer-milled flour particles decreased significantly (Table 3) with the decrease in the opening size of the mill screen (Table 2), which is consistent with the SEM images (Figure 2C and D). The volume-median diameter was reduced from 564 μm for 1 pass through the hammer mill with 1500 μm screen (HM1500P1) to 158 μm for 1 pass through the hammer mill with 500 μm screen (HM500P1). This was expected, as only the particles equal to or smaller than the opening size could travel through the mill screen. The volume-median diameter of the HM500P1 flour particles was significantly reduced, to 56 μm, with the second pass through the hammer mill using the same screen (HM500P2); the third pass (HM500P3), however, did not significantly change the volume-median diameter of the flour particles.

Starch Granule Damage. Both cryogenic and hammer milling processes increased the damage to starch granules (level 5 structure) as the size of the flour particles (level 6 starch structure) was reduced (Table 3) without significant effect on the starch content (~83% on the basis of dry-flour weight, Figure 1 in the Supporting Information). The damage to the starch granules in the cryogenically milled rice flours increased significantly from 4 to 15% when the total grinding time increased from 5 to 40 min (Table 1). For the hammer-milled rice flours, the damage to the starch granules increased significantly from 4 to 17% with the decrease in the opening size of the mill screen (Table 2). The damage to the starch granules of the HM500P1 flour increased significantly when the rice flour was hammer-milled with the same screen for the second pass (25%, HM500P2). Similar to the effects on the flour particle size, the third pass through the hammer mill with the same screen (HM500P3) did not significantly increase the damage to the starch granules.

At a similar degree of damage to the starch granules, the volume-median diameter of the hammer-milled rice flour particles was larger than that of the cryogenically milled rice flour counterparts (Table 3). Correspondingly, at a similar volume-median diameter, the damage to the starch granules was greater for the hammer-milled rice flour than the cryogenically milled counterpart.

Molecular Size Distributions of Fully Branched Starch. Starch mainly consists of amylopectin and amylose. Amylopectin is the larger glucan molecule (~10^8 Da) with a high number of short branches, whereas amylose is the smaller glucan molecule

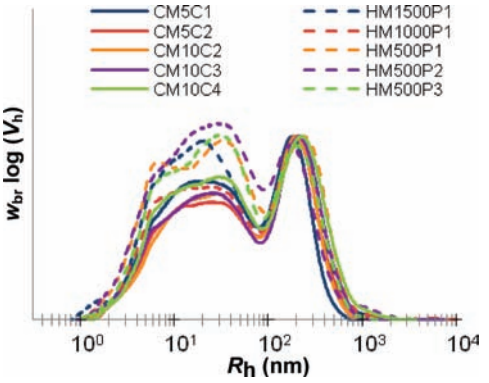

Figure 3. Weight molecular size distributions of fully branched starch in rice flours after different grinding treatments. The molecular size distributions are normalized to the same height of amylopectin peak (100 nm < R_h < 5,000 nm).

(~10^6 Da) with few long branches. The molecular size distributions of (whole) fully branched starch samples (Figure 3) are normalized to yield the same height of amylopectin peak (100 nm < R_h < 5,000 nm) to facilitate making inferences about amylopectin degradation into smaller molecules that might be coeluted with amylose (R_h < 100 nm).

The starch extraction/dissolution method developed by Syahariza et al.[18] allows for an accurate structure characterization of starch molecules (level 2 structure) in sorghum grains with minimal artifacts from the nonstarch components in the grains. The extraction/dissolution method, however, is not as effective in removing the nonstarch components of rice grains. It is likely caused by the high fraction of disulfide bonds in rice proteins reducing their solubility in ethanol and/or DMSO/LiBr solutions.[34] Thus, a treatment with sodium bisulfite solution, a reducing agent, was included in the extraction/dissolution method to facilitate the cleavage of the disulfide bonds. Figure 2 in the Supporting Information shows that the molecular size distribution of fully branched starch extracted from rice flour with the sodium bisulfite treatment had a reduced protein peak (R_h < 7 nm) compared with that without the treatment, making the protein peak less overlapping with the peaks of starch molecules, especially in the amylose region (R_h < 100 nm).

In principle, it would appear that knowing the size distribution of the protein by itself seemed to be useful in distinguishing the

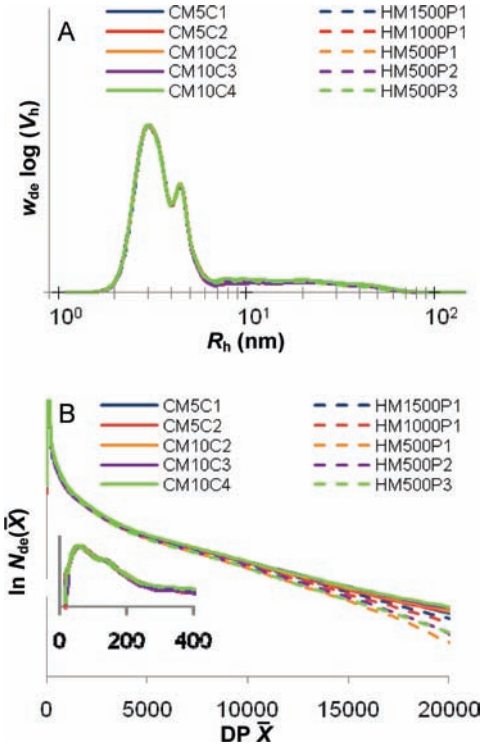

Figure 4. Molecular size distributions of debranched starch in rice flours after different grinding treatments; plotted as (A) debranched weight distribution, $w_{de}(\log V_h)$, and (B) debranched number distribution, $N_{de}(\bar{X})$. Inset in (B) is the debranched number distribution at smaller degrees of polymerization (DP). The molecular size distributions are normalized to the same height of the highest peak.

starch and protein components in the molecular size distributions. However, obtaining the molecular size distribution of isolated protein requires the assumption that the hydrodynamic radius of the protein does not change after protein extraction. The interaction among the rice protein molecules may be different from the interaction between a small amount of protein molecules and a large amount of starch molecules. Furthermore, protein extraction commonly involves various solvents, detergents, and/or salt solutions. All of these may change the intra- and intermolecular bonds in the proteins, resulting in different hydrodynamic size. A complete removal of protein is preferred so that the interaction between starch molecules and protein molecules can be avoided in the starch structure characterization. The improved dissolution technique developed in this study has been able to remove most of the proteins in rice grain: only two major peaks (amylose and amylopectin) were observed in the molecular size distribution of fully branched starch and the (minor) protein peak was almost unnoticeable.

The molecular size distributions of fully branched starch (level 2 structure) from all rice flours normalized to the same height of amylopectin peak are shown in Figure 3. All cryogenically milled flours showed similar molecular size distributions of fully branched starch, with apparent minor differences being within the variation of repeated SEC measurements of the same sample,[35] despite the differences in the flour particle size (level 6 structure) and the damage to the starch granules (level 5 structure) (Table 3). The molecular size distributions of fully branched starch from the hammer-milled rice flours, however, showed greater peak areas in the amylose region than those from the cryogenically milled rice flours, except that from the HM1000P1flour.

Molecular Size Distributions of Debranched Starch. The molecular size distributions of debranched starch (level 1 structure) from rice flours were normalized arbitrarily to yield the same height of the highest peak (Figure 4A and B). The first peak (1.5 nm < R_h < 4 nm) is of the amylopectin branches confined to one lamella (A and B1 chains), the second peak (4 nm < R_h < 6 nm) is of the amylopectin branches that span more than one lamella (B2, B3, ... chains), and the remaining broad peaks (6 nm < R_h < 100 nm) are the amylose branches.[26] The weight molecular size distributions of debranched starch, $w_{de}(\log V_h)$ (Figure 4A), and the number molecular size distributions of debranched starch, $N_{de}(X)$, at DP < 400 (Figure 4B inset) for all flour samples were superposable. There are thus no qualitative differences in the molecular size distributions of amylopectin branches despite the differences in the flour particle size (level 6 structure), the damage to the starch granules (level 5 structure), and the molecular size of the fully branched starch (level 2 structure) among the rice flours cryogenically milled and hammer-milled at various conditions (Table 3 and Figure 3). However, although it was not obvious in the weight molecular size distributions, $w_{de}(\log V_h)$, the ln $N_{de}(X)$ plot of molecular size of debranched starch, in general, showed greater amount of branches of DP > 10,000 in the cryogenically milled flours than in the hammer-milled flours (Figure 4B), suggesting that the cryogenically milled flours had more amylose branches than the hammer-milled flours.

DISCUSSION

The particle size of rice grains (level 6 starch structure) was rapidly reduced by both cryogenic- and hammer-milling processes until it reached a volume-median diameter of 56 μm or smaller (Table 3). Thereafter, the particle-size reduction of the rice grains by either milling process became insignificant. The smallest volume-median diameters between the two milling processes, however, were not significantly different, indicating that the particle size reduction of the rice grains was not dependent on the brittleness and toughness of the rice grains at the grinding temperatures used in this study.

Particle-size reduction of grains (level 6 structure) is commonly accompanied by the increase in the damage to starch granules (level 5 structure) caused by mechanical forces during grinding.[5−7,36] The damage to starch granules increased when the rice grains were cryogenically milled for a longer time (Table 3), which was attributed to the greater mechanical energy with the increase in grinding time. For the hammer-milled rice flours, the damage to starch granules increased when the opening size of the mill screen was decreased (Table 3). The mill screen is used to retain the rice-grain particles in the hammer mill until they are small enough to travel through the openings on the screen. Thus, the rice grains were ground for a longer time when they were ground in the hammer mill with the screen of smaller opening size, producing greater damage to the starch granules due to the increase in the mechanical energy at a longer grinding period.

The cryogenic-milling process damaged the starch granules (level 5 structure) in the rice flour to a lesser extent than the hammer-milling process when the rice grains were reduced to a similar volume-median diameter (level 6 structure) (Table 3). It has been reported that wheat bran underwent brittle fracture when it was ground at −80 °C or lower temperature; however, ductile fracture, which requires more mechanical energy, was

observed when the wheat bran was ground at −70 °C or higher temperature, ascribed to the increase in plastic behavior of the wheat bran.[22] The lesser damage to the starch granules by the cryogenic-milling process can be attributed to the increased brittleness of the rice grains with the decrease in the grinding temperature to cryogenic temperatures, increasing the grain fracturability and reducing the energy required to break the grain structure and to reduce the grain size.[20−22] Therefore, the cryogenically milled flours suffered less degradation caused by mechanical forces during grinding than the hammer-milled flours ground at ambient temperature.

The small particles of the cryogenically milled rice flours had jagged edges and corners (Figure 2E and F), whereas those of the hammer-milled flours had smooth surfaces and round corners (Figure 2G and H). The surface structure of the flour particles reflected the nature of the mechanical forces during grinding process.[16] The hammer mill pressed the grains against an abrasive ring at high rotating speed to break the grain structure, whereas the cryogenic mill in this study used less destructive impact energy to break the grains. This explains why the hammer-milled flours showed more effects of abrasion than the cryogenically milled flours.

The cryogenic milling of rice grains up to a total grinding time of 40 min did not affect the molecular size distribution of the fully branched starch (level 2 structure, Figure 3) despite the reduced particle size of the flour (level 6 structure) and the increased damage to starch granules (level 5 structure) (Table 3). This is in contrast with the increased cold-water solubility reported for the cryogenic milling of isolated starch granules, which was suggested to be the results of molecular degradation of amylopectin.[3] The molecular weight of isolated maize starch has also been reported to decrease with the increase in the damage to the starch granules.[36] The discrepancy could be attributed to the larger particle size of rice flour and grain compared with the isolated starch granules and/or the presence of the protein and/or cell-wall matrices in the rice flour, which is not the case in the isolated starch granules. The protein and/or cell-wall matrices surrounding the starch granules may provide protection against starch molecular degradation by absorbing the impact energy during cryogenic milling. Similar observations were reported when water was added to starch granules to a moisture level of 60% during cryogenic milling, reducing the damage to the starch granules and molecular degradation as indicated by the lesser increase in the cold-water solubility after grinding.[3] The protective effect of protein and/or cell-wall matrices, however, might be diminished when they were subjected to greater mechanical energy and/or more abrasive forces during hammer milling to break the tougher grain structure at higher grinding temperature.

The molecular size distribution of fully branched starch (level 2 structure) from the hammer-milled HM1500P1 flour showed a larger amylose peak area than that from the cryogenically milled CM5C1 flour (Figure 3) although the damage to the starch granules (level 5 structure) of the two flour samples were not significantly different (Table 3). This apparent discrepancy could be attributed to the relatively large particle size of the HM1500P1 flour compared with those of other flour samples (Figure 2 and Table 3). The starch granules in the large particles of the HM1500P1 flour might be entrapped in the protein and/or cell-wall matrices, preventing the complete dissolution of starch molecules in the DMSO/LiBr solution. Amylose is more readily extracted from the starch granules than amylopectin,[37] and thus incomplete dissolution of starch will result in an apparent larger amylose peak. Similar effects were not noticeable in the molecular size distribution of fully branched starch from the CM5C1 flour as it had finer particles than the HM1500P1 flour (Figure 2A and C, respectively, and Table 3). Furthermore, the molecular size distribution of fully branched starch in the HM1000P1 flour was not qualitatively different from those in the cryogenically milled flours. Syahariza et al.[18] has shown that the present dissolution method dissolves almost all of the starch molecules in cryogenically milled rice flour and the dissolution is not selective toward certain size populations of starch molecules. Thus, the results implied that complete starch dissolution with minimal molecular degradation was achieved for HM1000P1 and all cryogenically milled flours. In addition, the starch in the CM5C1 flour, which has the smallest damage to starch granules among all flour samples, should have the molecular structure closest to that of the unmilled starch; we note that it is impossible to analyze the starch structure from intact whole grain[17] and laboratory-scale wet-milling process commonly used to isolate starch granules with minimal damage to the starch granules has shown to cause molecular degradation.[18]

The larger amylose peak in the molecular size distributions of the fully branched starch (level 2 structure) from the HM500P1, HM500P2, and HM500P3 flours (Figure 3), together with their high damage to starch granules (level 5 structure, Table 3), suggests that molecular degradation of amylopectin occurred during hammer milling, producing smaller molecules that coeluted with amylose.[36] Molecular degradation of amylopectin has been reported after grinding or milling processes of grain or starch resulting in highly soluble, smaller molecules.[12,13] The results also suggest that the cleavage occurred in the inner part of the amylopectin molecules since the degraded amylopectin had a hydrodynamic size within the range of the hydrodynamic size of amylose, which is similar to the maximum stable size reported for degradation of starch molecules by extrusion[29] and by shear scission during elution through SEC column;[28] while the extrusion process is very different from that of milling, it would not be surprising if qualitatively similar effects are seen in both. If it were the outer branches of amylopectin, the hydrodynamic size of the degraded molecules would be smaller than amylose because individual amylopectin branches are much smaller than amylose molecules. It is not obvious from the current results if the molecular size distributions of fully branched starch in the HM500P2 and HM500P3 flours are qualitatively different as the difference is within the variation of repeated SEC measurements of the same sample.[35]

The correlations between the average hydrodynamic radius, $\bar{R}_h$, of fully branched molecules (level 2 structure) with the volume-median diameter of flour particles (level 6 structure) and the damage to starch granules (level 5 structure) are shown in Figure 5A and B, respectively. The $\bar{R}_h$ of HM1500P1 sample is excluded from the correlation because of the incomplete starch dissolution. There is no significant correlation between the particle size of rice flour and the average hydrodynamic radius of fully branched starch. However, a significant negative correlation is observed between the damage to starch granules and the average hydrodynamic radius of fully branched starch, which is expected as more molecules are degraded when the starch granules are more severely damaged.

There were no noticeable differences in the molecular size distributions of amylopectin branches (level 1 structure, Figure 4A and B inset) despite the differences in the flour particle size (level 6 structure), the damage to the starch granules (level 5 structure),

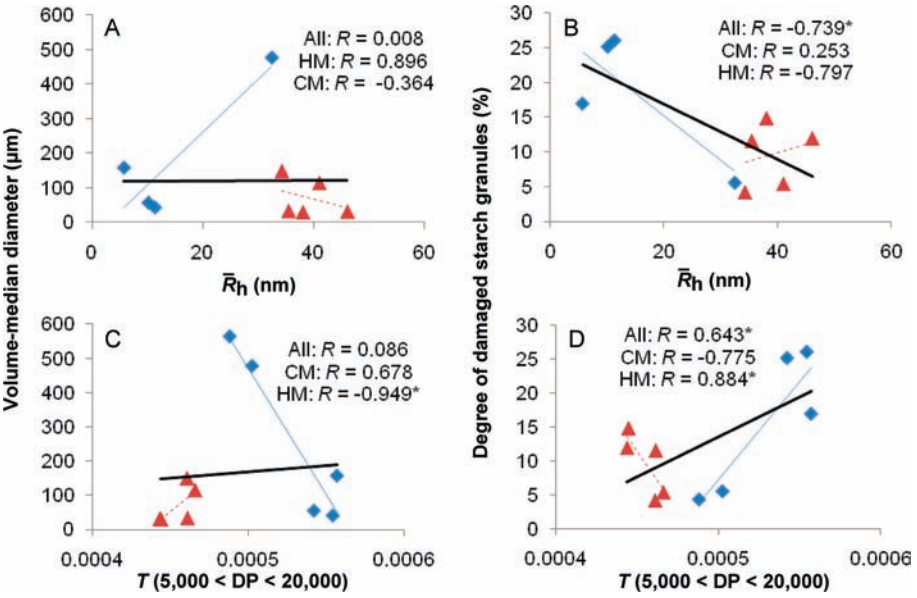

Figure 5. Correlations between average hydrodynamic radius ($\bar{R}_h$) from weight molecular size distributions of fully branched starch, $w_{br}(\log V_h)$, and slope (T) of number molecular size distributions of debranched starch, $N_{de}(\bar{X})$, for branches of DP between 5,000 and 20,000 with particle size and damage to the starch granules of cryogenically milled (▲, CM) and hammer-milled (◆, HM) rice flours. Thick black line is the correlation derived using all rice flour samples, broken red line is that using CM samples only, and thin blue line is that using HM samples only. * indicates significant difference at $p < 0.05$. The average hydrodynamic radius ($\bar{R}_h$) of HM1500P1 flour sample is excluded because of incomplete starch dissolution.

and the molecular size of the fully branched starch (level 2 structure) among the rice flours cryogenically milled and hammer-milled at various conditions (Table 3 and Figure 3). It has been reported that the molecular size distribution of amylopectin branches (level 1 structure) in native (unmilled) wheat starch was similar to that of the same starch after being ball-milled for 12 h, although the starch molecules (level 2 structure) were evidently degraded by the milling process.[13] The similarity in the molecular size distribution of amylopectin branches in the hammer-milled samples (level 1 structure, Figure 4A and B inset) might suggest that the degradation of amylopectin, as observed from the molecular size distribution of fully branched starch (level 2 structure, Figure 3), occurred predominantly at the α-1,6 glycosidic bonds located in the amorphous regions of the starch granules. Although it has been postulated that the glycosidic bonds in the amorphous regions are more susceptible to breakage by mechanical forces[13] because of the higher flexibility of the bonds in these regions, it is unlikely that the α-1,6 glycosidic bonds are more susceptible to the breakage than the α-1,4 glycosidic bonds, as the cleavage of α-1,4 glycosidic bonds has been reported for the amorphous (gelatinized) starch during extrusion.[29] A more likely scenario for the similar molecular size distribution of amylopectin branches despite the molecular degradation is that the amount of cleaved chains to result in the degradation as observed in the fully branched amylopectin molecules (level 2 structure) was minor compared with the overall number of amylopectin branches (level 1 structure); hence the molecular degradation observed from the HM500P1, HM500P2, and HM500P3 flours was not noticeable in their molecular size distributions of amylopectin branches (level 1 structure). The incomplete starch dissolution observed in the molecular size distribution of fully branched starch (level 2 structure, Figure 3) from the HM1500P1 flour was not noticeable in that of the amylopectin branches (level 1 structure, Figure 4A and B inset), which could be attributed to the increased solubility of the smaller amylopectin branches after debranching using isoamylase.

In general, the molecular size distributions of debranched starch from the cryogenically milled flours consistently showed more amylose branches of DP > 10,000 than those from the hammer-milled flours (level 1 structure, Figure 4B), showing that amylose degradation occurred during hammer milling of rice grains, but was not noticeable during cryogenic milling. This is consistent with the amylose degradation reported for extensive ball milling of isolated starches.[13,15] The results suggest that the milling process hammer is more likely to cleave longer branches; this is not unexpected, given that the longer chains suffer more mechanical forces during grinding as they span through more than one less-rigid amorphous lamella. The current results, however, are insufficient to shed light on the differences in the molecular size distributions of the longer branches among the starches in the hammer-milled flours, which do not follow the trends observed in the particle size or the damage to starch granules.

The slopes, T, of longer branches (5,000 < DP < 20,000) from the number distributions of debranched starch (level 1 structure), $N_{de}(\bar{X})$, from all flour samples were determined by plotting the number distributions as $\ln(N_{de}(\bar{X})/\bar{X})$ against $\bar{X}$ (Figure 3 in the Supporting Information). The correlations between T and the volume-median diameter of flour particles (level 6 structure) and the damage to starch granules (level 5 structure) are shown in Figure 5C and D, respectively. There are no significant correlations between T and the particle size of rice flours when all or only cryogenically milled flour samples are considered in the correlation test. A significant negative correlation is observed when only hammer-milled flour samples are considered in the correlation test, suggesting that the cleavage of the longer branches occurred when the grain size was reduced using hammer milling, but the cleavage of the longer branches was minimal during cryogenic milling. Significant positive

correlations between T and the damage to starch granules are observed when all or only hammer-milled flour samples are used in the correlation test, suggesting that more of the longer branches were cleaved when the starch granules were more severely damaged, especially during hammer milling.

The HM500P1, HM500P2, and HM500P3 flours suffered greater starch molecular degradation (level 1 and 2 structures) than the CM10C2, CM10C3, and CM10C4 flours (Figure 3), even though the volume-median diameters of the CM10C2, CM10C3, and CM10C4 flour particles (level 6 structure) were smaller (Table 3). Four possible mechanisms are proposed to explain this phenomenon: (1) Starch molecular degradation is only visible in the molecular size distribution of fully branched starch (level 2 structure) when the damage to starch granules (level 5 structure) equal to or greater than 17%; (2) cryogenic temperatures increase the fracturability of rice grains lowering the amount of energy required to reduce the flour particle size (level 6 structure) during grinding; (3) the abrasive forces during hammer milling are more destructive to starch molecular structure than the impact forces during cryogenic milling; and/or (4) protein and/or cell-wall matrices provide protection toward starch granules during cryogenic milling, but not hammer milling. More destructive means of grinding have also shown greater disruption of starch crystalline structure (level 3 structure) and higher cold-water solubility,[14−16] which might be related to the degradation of starch molecules (level 1 and 2 structures).

In conclusion, this study examined the changes in the flour particle size and structure (level 6 structure), the damage to starch granules (level 5 structure), the starch molecular structure (level 2 structure), and the individual branches of the molecules (level 1 structure) when polished rice grains were cryogenically milled and hammer-milled under various regimens. The size reduction of rice grains (level 6 structure) was accompanied by the increase in the damage to the starch granules (level 5 structure). Hammer milling produced greater damage to starch granules (level 5 structure) than cryogenic milling when the rice grains (level 6 structure) were reduced to a similar volume-median diameter. The starch molecular structure (level 1 and 2 structures) of the cryogenically milled rice flours was not evidently altered up to a total grinding time of 40 min despite the differences in the flour particle size (level 6 structure) and the damage to the starch granules (level 5 structure). On the other hand, molecular degradation of amylopectin and amylose (level 1 and 2 starch structures) was observed in the hammer-milled rice flours, especially those hammer-milled with 500 μm screen. The greater damage with hammer milling was also manifest in the preferential cleavage of longer branch chains. This has nutritional implications, because starches with longer branches are generally more slowly digested and thus more nutritionally desirable (e.g., ref 38). In addition, incomplete starch dissolution for structure characterization was observed for the rice flours hammer-milled with 1500 μm screen, which could be attributed to a large number of starch granules entrapped in the protein and/or cell-wall matrices of the large flour particles. At cryogenic temperatures, the rice grains were brittle and had increased fracturability, reducing the mechanical energy needed to break the grain structure and to reduce the grain size, and thus causing less degradation to the starch granules (level 5 structure) and molecules (level 1 and 2 structures) than at higher grinding temperatures.

These results show, for the first time, that the flour particle size (level 6 structure), the damage to the starch granules (level 5 structure), and the starch molecular structure (level 1 and 2 structures) can be independently altered by varying the grinding temperature and time, in addition to the types of grinding processes. Furthermore, the results also suggest that cryogenic milling can be used as the first step of starch extraction for accurate starch structure characterization (level 1 and 2 structures) and to reduce rice grain size and/or increase the damage to the starch granules (level 5 structure) without sacrificing the starch molecular structure (level 1 and 2 structures). The potential nutritional implications of these discoveries suggest that the effects of grinding on dietary fiber content and on digestibility indices such as glycemic index (which requires human subjects) should be implemented in the near future.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +61 7 3365 1865. Fax: +61 7 3365 1188. E-mail: j.hasjim@uq.edu.au.

Funding Sources

The authors thank the Australian Research Council (DP0985694, DP0986043 and LP0990769) for funding.

■ ACKNOWLEDGMENT

The authors thank Prof. Michael J. Gidley, Dr. Francisco Vilaplana, Mr. Torsten Witt, and Mr. Sushil Dhital for helpful discussions and Dr. H. Eustina Oh for assistance with the statistical analysis.

■ REFERENCES

(1) Oostergetel, G. T.; van Bruggen, E. F. J. The crystalline domains in potato starch granules are arranged in a helical fashion. *Carbohydr. Polym.* **1993**, *21* (1), 7–12.

(2) Gallant, D. J.; Bouchet, B.; Baldwin, P. M. Microscopy of starch: evidence of a new level of granule organization. *Carbohydr. Polym.* **1997**, *32* (3−4), 177–191.

(3) Dhital, S.; Shrestha, A. K.; Gidley, M. J. Effect of cryo-milling on starches: Functionality and digestibility. *Food Hydrocolloids* **2010**, *24* (2−3), 152–163.

(4) Hasjim, J.; Srichuwong, S.; Scott, M. P.; Jane, J.-l. Kernel composition, starch structure, and enzyme digestibility of *opaque-2* maize and quality protein maize. *J. Agric. Food. Chem.* **2009**, *57* (5), 2049–2055.

(5) Nowakowski, D.; Sosulski, F. W.; Hoover, R. The effect of pin and attrition milling on starch damage in hard wheat flours. *Starch/Staerke* **1986**, *38* (8), 253–258.

(6) Hatcher, D. W.; Anderson, M. J.; Desjardins, R. G.; Edwards, N. M.; Dexter, J. E. Effects of flour particle size and starch damage on processing and quality of white salted noodles. *Cereal Chem.* **2002**, *79* (1), 64–71.

(7) Nishita, K. D.; Bean, M. M. Grinding methods: Their impact on rice flour properties. *Cereal Chem.* **1982**, *59* (1), 46–49.

(8) Dexter, J. E.; Preston, K. R.; Martin, D. G.; Gander, E. J. The effects of protein content and starch damage on the physical dough properties and bread-making quality of Canadian durum wheat. *J. Cereal Sci.* **1994**, *20* (2), 139–151.

(9) Collins, T. H. The importance of damaged starch. *Milling* **1971**, *153*, 17–20.
(10) Lelievre, J. Starch damage. *Starch/Staerke* **1974**, *26* (3), 85–88.
(11) Tester, R. F. Properties of damaged starch granules: Composition and swelling properties of maize, rice, pea and potato starch fractions in water at various temperatures. *Food Hydrocolloids* **1997**, *11* (3), 293–301.
(12) Stark, J. R.; Yin, X. S. The effect of physical damage on large and small barley starch granules. *Starch/Staerke* **1986**, *38* (11), 369–374.
(13) Morrison, W. R.; Tester, R. F. Properties of damaged starch granules. IV. Composition of ball-milled wheat starches and of fractions obtained on hydration. *J. Cereal Sci.* **1994**, *20* (1), 69–77.
(14) Morrison, W. R.; Tester, R. F.; Gidley, M. J. Properties of damaged starch granules. II. Crystallinity, molecular order and gelatinisation of ball-milled starches. *J. Cereal Sci.* **1994**, *19* (3), 209–217.
(15) Yin, X. S.; Stark, J. R. Molecular modification of barley starch granules by different types of physical treatment. *J. Cereal Sci.* **1988**, *8* (1), 17–28.
(16) Becker, A.; Hill, S. E.; Mitchell, J. R. Milling—A further parameter affecting the rapid visco analyser (RVA) profile. *Cereal Chem.* **2001**, *78* (2), 166–172.
(17) Gidley, M. J.; Hanashiro, I.; Hani, N. M.; Hill, S. E.; Huber, A.; Jane, J.-L.; Liu, Q.; Morris, G. A.; Rolland-Sabaté, A.; Striegel, A. M.; Gilbert, R. G. Reliable measurements of the size distributions of starch molecules in solution: Current dilemmas and recommendations. *Carbohydr. Polym.* **2010**, *79* (2), 255–261.
(18) Syahariza, Z. A.; Li, E.; Hasjim, J. Extraction and dissolution of starch from rice and sorghum grains for accurate structural analysis. *Carbohydr. Polym.* **2010**, *82* (1), 14–20.
(19) Gujral, H. S.; Rosell, C. M. Improvement of the breadmaking quality of rice flour by glucose oxidase. *Food Res. Int.* **2004**, *37* (1), 75–81.
(20) Singh, K. K.; Goswami, T. K. Design of a cryogenic grinding system for spices. *J. Food Eng.* **1999**, *39* (4), 359–368.
(21) Goswami, T. K.; Singh, M. Role of feed rate and temperature in attrition grinding of cumin. *J. Food Eng.* **2003**, *59* (2−3), 285–290.
(22) Hemery, Y. M.; Mabille, F.; Martelli, M. R.; Rouau, X. Influence of water content and negative temperatures on the mechanical properties of wheat bran and its constitutive layers. *J. Food Eng.* **2010**, *98* (3), 360–369.
(23) Mahasukhonthachat, K.; Sopade, P. A.; Gidley, M. J. Kinetics of starch digestion in sorghum as affected by particle size. *J. Food Eng.* **2010**, *96* (1), 18–28.
(24) AACC International Method 76-31.01, Determination of Damaged Starch—Spectrophotometric Method. Final approval November 8, 1995; Reapproval November 3, 1999. In *Approved Methods of Analysis*, 11th ed.; AACC International: St. Paul, MN, U.S.A.
(25) AACC International Method 76−13.01, Total Starch Assay Procedure (Megazyme Amyloglucosidase/α-Amylase Method). First approval November 8, 1995; Reapproval November 3, 1999. In *Approved Methods of Analysis*, 11th ed.; AACC International: St. Paul, MN, U.S.A.
(26) Hasjim, J.; Cesbron Lavau, G.; Gidley, M. J.; Gilbert, R. G. *In vivo* and *in vitro* starch digestion: Are current *in vitro* techniques adequate?. *Biomacromolecules* **2010**, *11* (12), 3600–3608.
(27) Witt, T.; Gidley, M. J.; Gilbert, R. G. Starch digestion mechanistic information from the time evolution of molecular size distributions. *J. Agric. Food. Chem.* **2010**, *58* (14), 8444–8452.
(28) Cave, R. A.; Seabrook, S. A.; Gidley, M. J.; Gilbert, R. G. Characterization of starch by size-exclusion chromatography: The limitations imposed by shear scission. *Biomacromolecules* **2009**, *10* (8), 2245–2253.
(29) Liu, W.-C.; Halley, P. J.; Gilbert, R. G. Mechanism of degradation of starch, a highly branched polymer, during extrusion. *Macromolecules* **2010**, *43* (6), 2855–2864.
(30) Vilaplana, F.; Gilbert, R. G. Characterization of branched polysaccharides using multiple-detection size separation techniques. *J. Sep. Sci.* **2010**, *33* (22), 3537–3554.
(31) Clay, P. A.; Gilbert, R. G. Molecular weight distributions in free-radical polymerizations. 1. Model development and implications for data interpretation. *Macromolecules* **1995**, *28* (2), 552–569.
(32) Jane, J.-L.; Kasemsuwan, T.; Leas, S.; Zobel, H.; Robyt, J. F. Anthology of starch granule morphology by scanning electron microscopy. *Starch/Staerke* **1994**, *46* (4), 121–129.
(33) Chiou, H.; Fellows, C. M.; Gilbert, R. G.; Fitzgerald, M. A. Study of rice starch structure by dynamic light scattering in aqueous solution. *Carbohydr. Polym.* **2005**, *61* (1), 61–71.
(34) Paraman, I.; Hettiarachchy, N. S.; Schaefer, C.; Beck, M. I. Hydrophobicity, solubility, and emulsifying properties of enzyme-modified rice endosperm protein. *Cereal Chem.* **2007**, *84* (4), 343–349.
(35) Hoang, N.-L.; Landolfi, A.; Kravchuk, A.; Girard, E.; Peate, J.; Hernandez, J. M.; Gaborieau, M.; Kravchuk, O.; Gilbert, R. G.; Guillaneuf, Y.; Castignolles, P. Toward a full characterization of native starch: Separation and detection by size-exclusion chromatography. *J. Chromatogr., A* **2008**, *1205* (1−2), 60–70.
(36) Chen, C.-J.; Shen, Y.-C.; Yeh, A.-I. Physico-chemical characteristics of media-milled corn starch. *J. Agric. Food. Chem.* **2010**, *58* (16), 9083–9091.
(37) Srichuwong, S.; Sunarti, T. C.; Mishima, T.; Isono, N.; Hisamatsu, M. Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties. *Carbohydr. Polym.* **2005**, *62* (1), 25–34.
(38) Li, L.; Jiang, H.; Campbell, M.; Blanco, M.; Jane, J. Characterization of maize amylose-extender (*ae*) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. *Carbohydr. Polym.* **2008**, *74* (3), 396–404.