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Wet ball milling of *indica* rice starch effectively modifies its multi-level structures and pasting behavior

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Abstract

While the changes in starch physicochemical properties resulting from typical dry ball milling (usually requiring hours) have been widely studied, there has been limited knowledge regarding how wet ball milling (with liquid media) affect starch structure and properties. This work was to investigate the effect of wet ball milling on the multi-scale structures and pasting behavior of *indica* rice starch. For this starch, increasing ball-milling time resulted in decreases in particle size, crystallinity, and double helix content and increases in single helix and amorphous contents. Only 15 min of ball milling effectively destroyed the semi-crystalline lamellar structure and resulted in the cleavage of long chains from amylose and amylopectin backbones and marked decreases in pasting temperature and viscosity, while ball milling for even longer duration did not cause significant changes in these aspects. Thus, this work shows the high efficacy of wet ball milling for modifying rice starch structure and properties.

Keywords: Starch mechanical treatment; Wet ball milling; Starch multi-scale structure; Lamellar structure; Molecular structure; Pasting properties

1 Introduction

Starch is widely found in plants such as potato, corn, wheat, and rice.¹⁻² As a major food ingredient that provides energy for humans, starch has aroused significant interest in the development of food products with health benefits.³⁻⁴ Starch has also been studied as a drug-delivery carrier material.⁵⁻⁶ However, native starch usually does not fully meet the application requirements.⁷ To improve its physicochemical properties, starch needs to be modified to generate higher-value products.⁸ Biological methods such as enzyme treatment have been used to produce porous starch and low-molecular-mass products such as syrup and maltodextrin.⁹⁻¹⁰ Chemical methods can be used to improve emulsifiability, change gelatinization behavior, and reduce hydrophilicity for starch.¹¹⁻¹³ In recent years, people have paid great attention to physical modification methods such as microwave radiation, sonication, and mechanical milling.¹⁴⁻¹⁶

Among different physical modification methods, ball milling is a simple method to generate micronized starch with modified physicochemical properties. The mechanical forces provided by ball milling has been widely found to result in changes in starch structure and properties such as, granule morphological change¹⁷ and agglomeration (cassava,¹⁸ cassava and Peruvian carrot),¹⁹ increases in apparent amylose content (cassava and maize),²⁰ cold-water solubility (cassava,¹⁸ cassava and maize,²⁰ cassava and Peruvian carrot,¹⁹ maize),²¹ water absorption (cassava and Peruvian carrot),¹⁹ transparency (cassava and maize,²⁰ maize),²¹ and syneresis (maize),²¹ and decreases in crystallinity (cassava,¹⁸ cassava and Peruvian carrot),¹⁹ gelatinization temperature and enthalpy change (cassava and maize,²⁰ cassava and Peruvian carrot),¹⁹ viscosity (cassava,¹⁸ cassava and Peruvian carrot),¹⁹ and shear thinning (cassava).¹⁸ Tan et al.²² reported that ball-milled waxy maize starch showed reduced pasting temperature and paste viscosity, increased pasting stability, and a reduced tendency to retrogradation, whereas the same treatment caused no significant changes to the structure and properties of high-amylose starch.

The effect of ball milling on the structure and properties of rice starch has also been studied but not as extensively as for other types of starch. According to Devi et al.,²³ cryo-milled rice starch showed decreased crystallinity, pasting temperature, and viscosity and increased water solubility and water absorption. Zhang et al.²⁴ found that ball milling changed the shape of rice granules from polyhedron to anomalous shapes, destroyed the starch crystalline structure, decomposed both amylopectin and amylose, increased cold-water solubility and reducing power, and decreased pasting temperature and viscosity. González et al.²⁵ reported that, for rice starch, increasing milling energy led to reduced particle size, crystallinity degree, and gelatinization enthalpy. Soe et al.²⁶ indicated that, for Thai glutinous rice starch, there was a reduction in crystallinity after ball milling, which could be associated with changes in cold water solubility, swelling capacity, and gelatinized dispersion viscosity, expanding its application as a mucoadhesive polymer. Nonetheless, though *indica* rice is an important rice cultivar in Asia, how ball milling, especially with wet media, changes the multiscale structures of *indica* rice starch has been scarcely been studied. The effect of wet ball milling on other types of starch has been reported to a limited extent²⁷ but not on rice starch, to the best of our knowledge. Compared with dry ball milling, wet ball milling, with aqueous media involved, is more energy-efficient, provides better heat dissipation, avoids dead corners, and produces smaller particles. The higher efficiency of wet ball milling could be due to the action of the aqueous solution as the grinding medium, which facilitates the increasing and deepening of cracks and the fragmentation of the ground material. Moreover, wet ball milling treats solid suspensions (which is in line with many processes for starch or starch-based foods) and avoids dust.

In this work, *indica* rice starch was subjected to different durations of wet ball milling, by which micronized starch of varied granule size was obtained. The multilevel structures (i.e. granule, lamellar, crystalline, short-range order, and molecular) and pasting properties were studied, which were correlated with each other. The knowledge generated from this work could provide an insight

into the development of modified rice starch products by “green” cost-effective mechanical treatment.

2 Materials and methods

2.1 Materials

Indica rice from Xiangyang Saiya Rice Co., Ltd (Xiangyang, China) was used to extract rice starch. The rice was soaked in distilled water at a ratio of 1:3 (w/v) for 3 h at room temperature and then ground into a slurry with a laboratory GM-WZ150 colloid mill (Shishou, China) for 3 min. The slurry was centrifuged (SF-TDL-5A, Shanghai Feiqiaer Analytical Instrument Co., Ltd, Shanghai, China) at 4000 rpm for 5 min, and dried at 45 °C overnight to obtain *indica* rice flour. The rice flour was soaked in 0.2% w/v NaOH solution (1:3, w/w), stirred for 1 h, and centrifuged at 4000 rpm for 3 min. This procedure was performed four times, and in the last-time, centrifugation was performed at 4000 rpm for 15 min. The rice flour was suspended in distilled water (1:2, w/v), stirred for 10 min, neutralized with 0.1 mol/L HCl to pH 7, and centrifuged at 4000 rpm for 8 min, which was performed twice. The product was then stirred with 95% ethanol (1:2, w/v) for 10 min, and centrifuged at 4000 rpm, followed by drying at 40 °C for 12 h to obtain *indica* rice starch.²⁸

2.2 Preparation of ball-milled starch

The ball milling of starch was conducted using a MINI ZETA 03E high-energy wet ball mill (Netzsch-Gerätebau GmbH, Germany). For this experiment, 40 g of the starch was placed into the container of the ball mill with 400 mL of water. Then, 740 g of zirconia balls were added into the container. The sample mixture was treated at an agitation speed of 2500 rpm for different durations (i.e. 15, 30, 60, and 90 min). The starch slurries after ball milling were measured by laser diffraction analysis (detailed in the next section) to examine the changes in particle size of starch during milling. In addition, the slurries were dried at 40 °C in an oven overnight and ground for further analyses.

2.3 Laser diffraction analysis

The particle size distribution of starch granules was measured using a laser diffraction analyzer (Malvern, UK) with a flow cell of 1000 mL. The sample was added into a reservoir and dispersed in distilled water to achieve a shading value of 12% to 17%. The pumping speed was set to be 2050 rpm. All results are reported as the average based on three replicates.

2.4 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) imaging was undertaken on a JEOL JSM-6460LA scanning electron microscope (Tokyo, Japan) at 5 kV. Starch granules were mounted on a metal stage and then sputter-coated with platinum.

2.5 Small-angle X-ray scattering (SAXS)

Small-angle X-ray scattering (SAXS) testing was performed on a NanoSTAR system (Bruker, USA) at 30 W with CuK α radiation as the X-ray source. For this measurement, starch slurries with a water content of 60 wt% were equilibrated at 20 °C for 12 h. Each starch slurry was placed in a sample cell and exposed to an incident X-ray monochromatic beam for 15 min. Data was recorded using a VÅnTeC-2000 detector and the scattering data for the sample pool with water was used as the background. The data over the range of $0.08 < q < 2.00 \text{ \AA}^{-1}$ were used. The scattering vector q (\AA^{-1}) is equal to $4\sin\theta/\lambda$, in which 2θ is the scattering angle.²⁹⁻³⁰

2.6 X-ray diffraction (XRD)

The crystalline structure of starch was evaluated using a Bruker D8 Advance X-ray diffractometer (USA) at 40 kV and 30 mA. The XRD patterns were obtained with a 2θ range of 4–40°, a step size of 0.02°, and a step rate of 0.5 s/step.

2.7 Nuclear magnetic resonance (NMR) spectroscopy

Solid-state cross-polarization magic-angle spinning carbon-13 nuclear magnetic resonance (^{13}C CP/MAS NMR) spectroscopy was performed using an Advance AV spectrometer (Bruker, USA) with a 4-mm wideband dual-resonance MAS probe. Approximately 500 mg of the starch sample was placed in a rotator which was inserted into the center of the magic field. NMR spectra were recorded at a temperature of 295 K and a frequency of 100.613 MHz. For spectra with a recirculation delay of 2 s, at least 3000 scans were accumulated. Spectra were analyzed using PeakFit software version 4.12 to calculate the content of single helices and double helices in the starch.³¹⁻³²

2.8 Size-exclusion chromatography (SEC)

For size-exclusion chromatography (SEC) analysis, each starch was dissolved in a DMSO/LiBr solution consisting of 99.5% DMSO and 0.5% LiBr. The starch content in DMSO/LiBr was determined by a Megazyme Total Starch Assay Kit and adjusted to 2 mg/mL prior to SEC analysis. Then, the molecular size distribution of the starch was measured using an Agilent 1100 SEC system with a Shimadzu RID-10A refractive index detector (Shimadzu Corporation, Kyoto, Japan) based on an earlier method³³⁻³⁴ with modification. GRAM pre-columns and GRAM 100 and 3000 columns (PSS, Germany) were used and maintained at a constant 80 °C. The DMSO/LiBr solution was used to elute starch molecules during the test. The elution volume was converted to a hydrodynamic volume V_h (or corresponding a hydrodynamic radius R_h).³³

Also, the chain length distributions of debranched starches were measured using our earlier method.³⁵ The solutions of starch in DMSO/LiBr had a starch concentration of about 2 mg/mL. The starch molecules were fully debranched using isoamylase³⁶⁻³⁷ and were used for the testing. The starch solution samples were measured using an SEC system (Agilent 1100 Series) with GRAM precolumn, GRAM 100 and GRAM 1000 columns (PSS, Germany). The flow rate was set at 0.6

mL/min. For these debranched starch, the hydrodynamic volume (V_h) was converted into degree of polymerization (DP) based on the Mark–Houwink equation.³⁸

2.9 Rapid visco analysis

A rapid visco analyzer (RVA) (RVA-TecMaster, Perten, Sweden) was used to study the pasting behavior of ball-milled starch samples. About 1.5 g of starch was placed in the sample canister containing 25 g of distilled water. The starch was stirred in the water by sliding up and down and rotating the RVA impeller, immediately before the RVA canister and the impeller were mounted onto the RVA and testing. The test profile consisted of heating from 50 °C to 95 °C, keeping at 95 °C, and then cooling and holding at 50 °C, as described previously.³⁹ The total test time was 750 s (12.5 min)

2.10 Statistical analysis

Data were expressed as mean \pm standard deviation (SD) and were statistically analyzed by using IBM SPSS software version 20.0 (Chicago, IL, USA). A statistical difference at $P < 0.05$ was considered to be significant.

3 Results and discussion

3.1 Granule size distribution and morphology

Fig. 1 shows the particle size distribution of rice starch wet-ball-milled for different durations. For the original rice starch, the size distribution displayed two peaks, with a sharp one centered on about 6 μm and another small one centered on 1 μm . For the 15-min and 30-min ball-milled samples, the size distribution showed predominantly a single-peak centered on about 6 μm and 5 μm , respectively. For the rice starch wet-ball-milled for 60–90 min, the main peak of size distribution moved to lower values and there was an additional peak centered on about 0.2 μm . For these samples,

a longer time of wet ball milling resulted in a shift of the major peak position to lower particle size, a decrease in the intensity of the major peak, and an increase in the size of the secondary peak.

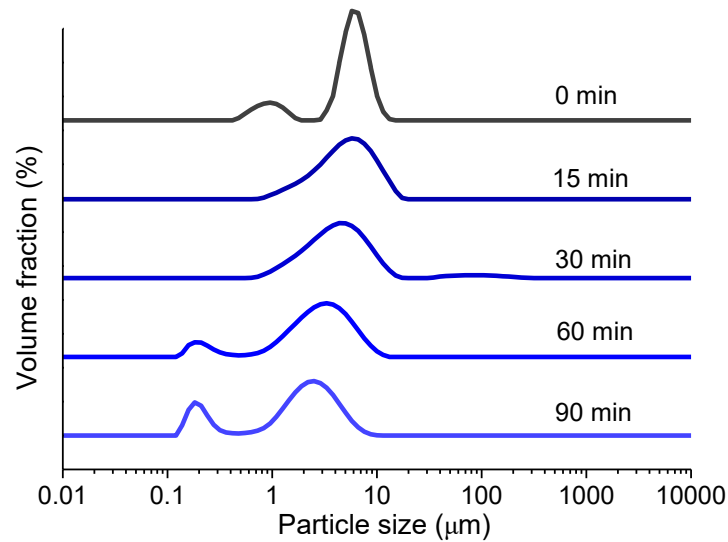


Fig. 1 Effect of wet ball milling duration on the particle size distribution of rice starch

Table 1 lists the particle size parameters of wet-ball-milled rice starch. The $D_{(0.5)}$ value (the diameter value less than which 50% of the overall particles have) of the untreated starch was 6.74 μm , indicating that this rice starch is a small-granule starch. As the milling time increased, $D_{(0.5)}$ decreased gradually, which is consistent with previous reports.^{18, 24} Correspondingly, surface-area-average particle size and volume-average particle size also showed a decreasing trend for the duration of testing (90 min).

Table 1 Particle size parameters of rice starch wet-ball-milled for different durations.

Ball milling duration (min)	SAAPS (μm)	VAPS (μm)	$D_{0.1}$ (μm)	$D_{0.5}$ (μm)	$D_{0.9}$ (μm)
0	2.58 ± 0.03^c	7.58 ± 0.72^b	1.09 ± 0.01^c	6.74 ± 0.01^a	12.42 ± 0.19^a
15	4.22 ± 0.01^a	6.11 ± 0.07^c	2.17 ± 0.01^a	5.50 ± 0.02^b	10.97 ± 0.11^b

30	3.62±0.05 ^b	9.67±2.17 ^a	1.82±0.01 ^b	4.59±0.05 ^c	10.60±0.62 ^b
60	1.21±0.00 ^d	3.36±0.01 ^d	0.36±0.00 ^d	2.97±0.01 ^d	6.51±0.01 ^c
90	0.74±0.00 ^g	2.34±0.00 ^{de}	0.21±0.00 ^g	2.15±0.01 ^g	4.65±0.02 ^e

SAAPS, Surface-area-average particle size; VAP, volume-average particle size; Values are means of three determinations. The different superscript letter within a row indicates significant difference ($P < 0.05$).

Fig. 2 shows the SEM images of *indica* rice starch wet-ball-milled for different durations. The original rice starch without ball milling (0 min) was an irregular polyhedron⁴⁰ with sharp edges and corners, and the granule surface was flat without cracks. After wet ball milling, even for only 15 min, the morphology of starch changed significantly — starch granules were mainly in the form of agglomerations. With regard to this, the wet-ball-milled starch (broken granules and leached molecules) could aggregate together during dehydration. Similarly, Huang et al.¹⁸ reported the agglomeration of cassava starch granules after ball milling. No significant further change to this morphology can be seen with prolonged duration (up 90 min) of wet ball milling.

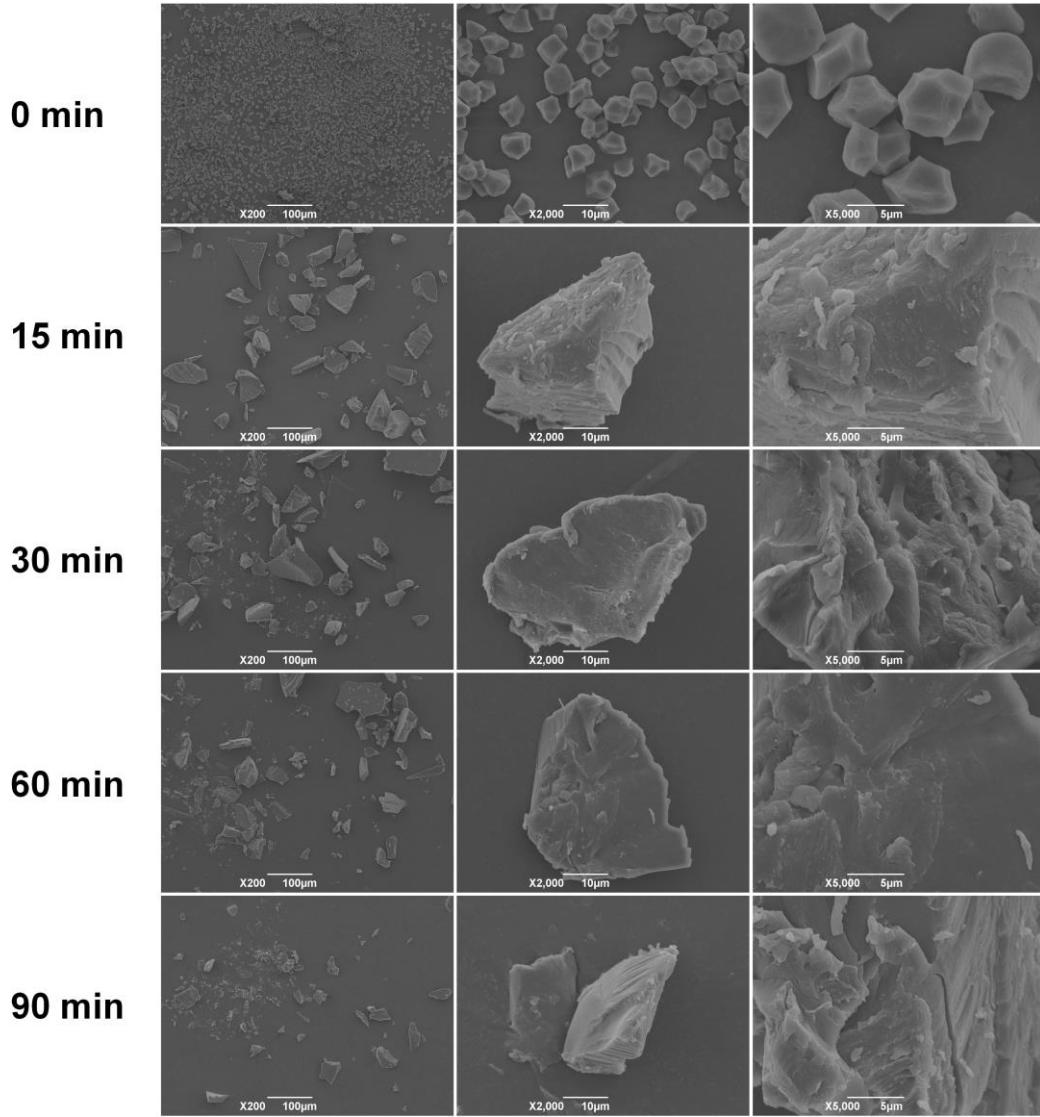


Fig. 2 SEM images of starch granules wet-ball-milled for different durations.

3.2 Nanostructure

Fig. 3 shows the SAXS curves for rice starch wet-ball-milled for different durations. The original rice starch showed a SAXS peak in the range of $0.06\text{--}0.07\text{ \AA}^{-1}$, reflecting semi-crystalline lamellae of 9–10 nm thickness based on Woolf-Bragg's equation $d = 2\pi/q$.^{41–42} It has been shown that higher peak intensity for starch could be associated with a greater difference in electron density between amorphous and crystalline lamellae.⁴³ For rice starch wet-balled-milled, even for only 15 min, the major peak was absent, suggesting wet ball milling effectively destroyed the lamellar

structure of rice starch. The starch with wet ball milling for 15 min exhibited a broad inflection at q values (about 0.03 to 0.06 \AA^{-1}) lower than the position of the lamellar peak. This inflection is associated with larger aggregates of starch chains on the nanoscale,⁴⁴ from the disrupted lamellae or other types of structure such as long-range polymorphs. This inflection displayed slight changes as the processing time increased up to 90 min.

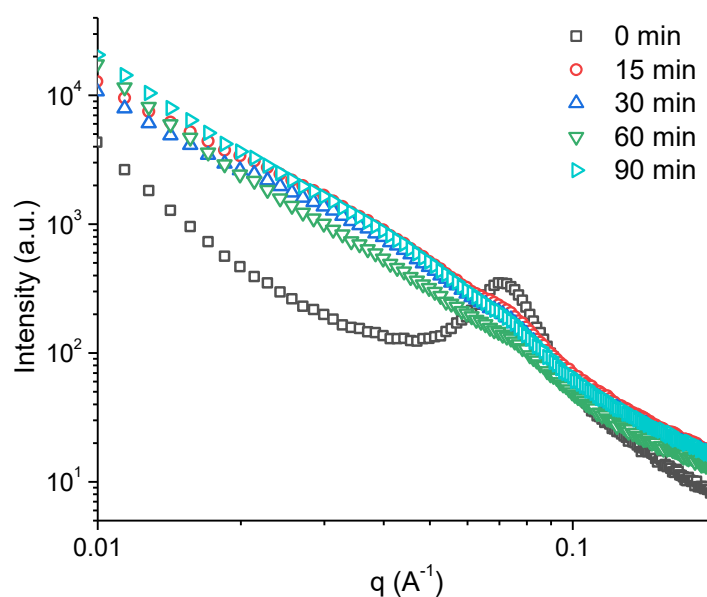


Fig. 3 Small-angle X-ray scattering (SAXS) curves for rice starch wet-ball-milled for different durations.

3.3 Crystalline structure

Fig. 4 shows the XRD curves for rice starch wet-ball-milled for different durations. For the original rice starch, there were strong diffraction peaks at $2\theta = 15.2^\circ$ and 23.2° along with a doublet at 17.3° and 18.0° , which are characteristic of the A-type crystalline structure.⁴⁵⁻⁴⁶ Also, there were diffractions of V-type crystallites such as that at about 20° 2θ . Wet-ball-milled starch samples displayed the same XRD pattern but with significantly decreased intensity, indicating that the mechanical treatment disrupted the starch crystalline structure effectively. A reduction in

crystallinity induced by ball milling for other starches was also reported.¹⁸⁻¹⁹ There were no significant differences in the XRD pattern among samples with different durations of wet ball milling, suggesting 15 min was enough to reach the maximal change to the crystalline structure.

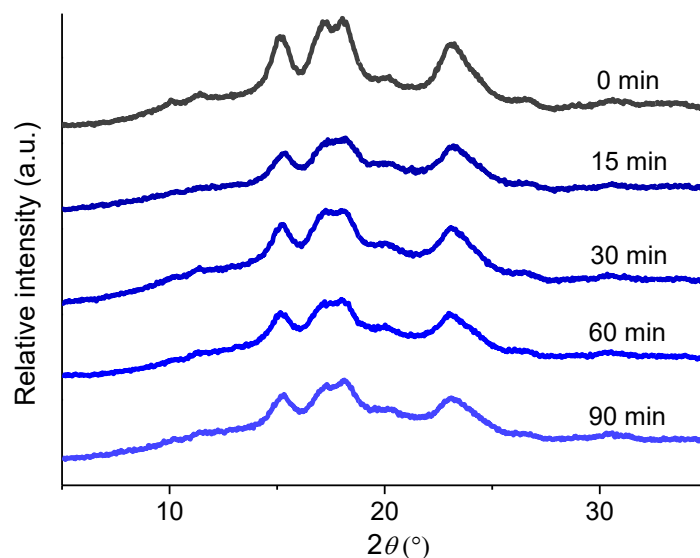


Fig. 4 X-ray diffractograms for rice starch wet-ball-milled for different durations.

3.4 Short-range order

The solid-NMR spectra and their sub-spectra of wet-ball-milled starches are included in **Fig. 5**. In an NMR spectrum for starch, the C1 (93–103 ppm) signal contains information relating to the ordered and amorphous regions of starch. In general, A-type starch has a double-helical conformation that adopts a twofold packing symmetry resulting in three inequivalent residues per turn, and the C1 region has three overlapping peaks at about 103, 101, and 100 ppm. For B-type starch, the threefold symmetry of adjacent helices leads to two inequivalent residues per turn, and the C1 region has two overlapping peaks at about 103 and 101 ppm.³² For rice starch as an A-type starch, there is a triplet mode in the C1 region and the main resonance signal for C1 is at 103 ppm, that for C2,3,5 at 73 ppm, that for C4 at 82 ppm, and that for C6 at 62 ppm, as shown in **Table 2**. There was no significant change in the ¹³C chemical shift between the original rice starch and wet-ball-milled

rice starch. For the latter, the C1 triplet for the original starch became blurred, and the peaks for amorphous starch became more intense relative to those for ordered parts, indicating that some crystalline structure of starch changed into an amorphous state under the action of mechanical force. This result corresponds to the XRD result.

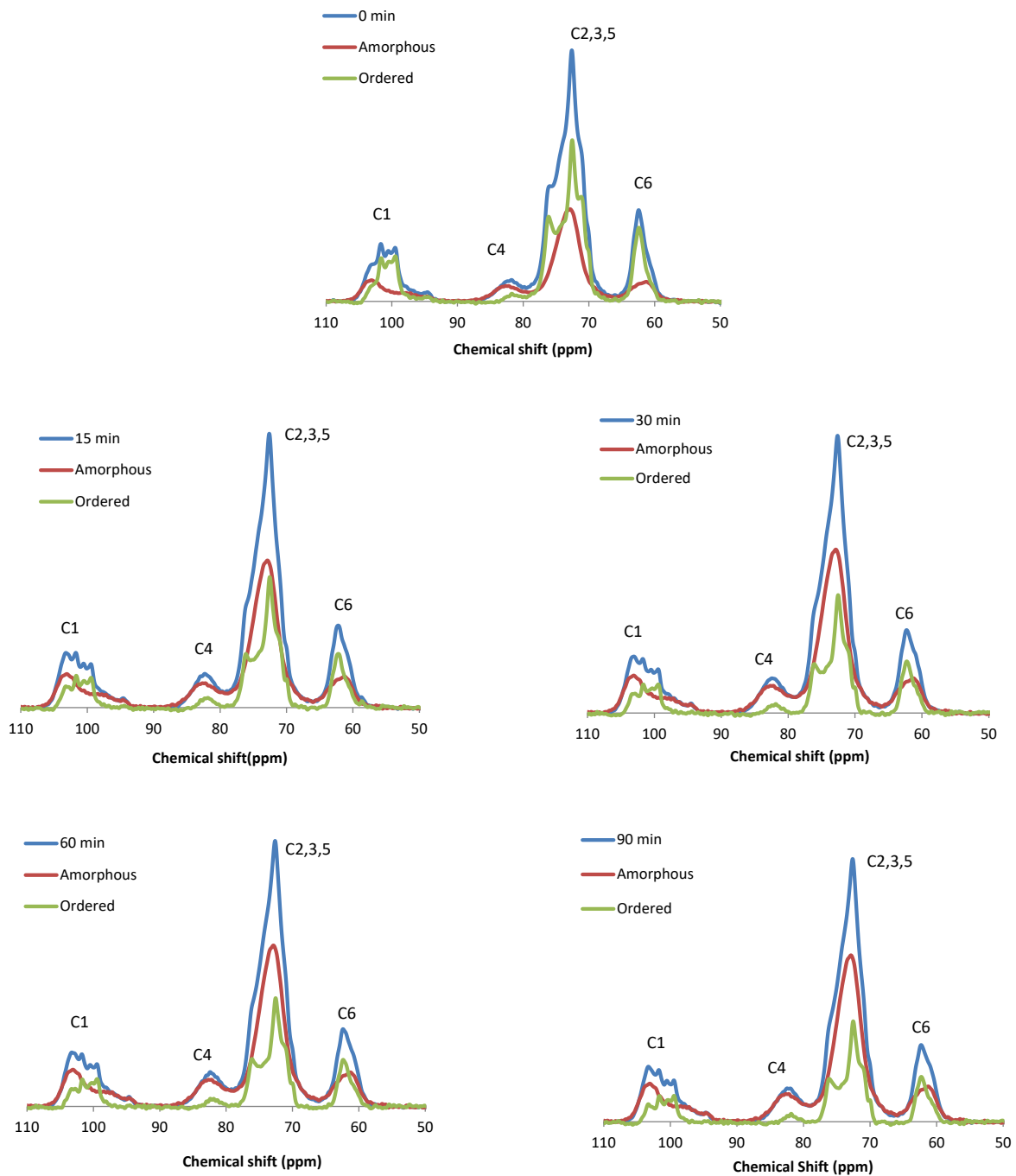


Fig.5 ^{13}C CP/MAS NMR spectra and their ordered sub-spectra for rice starch wet-ball-milled for different durations.

Table 2 Peak positions of the deconvoluted peaks, aligned to different carbon atoms in the glucose ring, of the ordered sub-spectra of NMR for rice starch wet-ball-milled for different durations.

Ball milling	V-type	A-type		
duration (min)	C1	C1	C2,3,4,5	C6
0	103.0	99.7, 100.6, 101.5	71.4, 72.6, 73.9, 76.0	62.3
15	103.2	99.4, 100.5, 101.7	72.4, 74.6, 76.0, 81.9	62.1
30	103.0	99.7, 100.7, 101.5	72.3, 72.6, 75.8, 82.0	62.1
60	103.0	99.7, 100.6, 101.5	72.3, 72.6, 75.8, 81.8	62.1
90	103.2	99.6, 100.6, 101.6	72.3, 74.7, 76.0, 81.8	62.1

To quantify the evolution of the short-range order of starch, **Table 3** shows the relative proportions of single-helices and double-helices in wet-ball-milled rice starch samples. A general trend is that, with longer duration of wet ball milling, the proportion of amorphous starch increased, that of single helices increased moderately, and that of double helices decreased. These results indicate that wet ball milling effectively disrupted double helices, and some starch chains formed single helices under this treatment.

Table 3 Relative proportions of amorphous, single, and double-helix conformations in rice starch wet-ball-milled for different durations.

Ball milling	Amorphous (%)	Single helix (%)	Double helix (%)
duration (min)			
0	47.00	9.47	43.53

15	58.01	10.01	31.98
30	63.47	10.98	25.55
60	65.21	11.39	23.40
90	67.03	9.32	23.65

3.5 Molecular size distribution

Fig. 6a shows the molecular size distribution of rice starch wet-balled-milled for different durations. The original rice starch displayed two peaks near hydraulic radii (R_h) of 100 nm and 20 nm, mainly from larger-molar-mass amylopectin and smaller-molar-mass amylose or small branched amylopectin composition.⁴⁷ Among them, the peak of R_h near 100 nm was much more prominent than that near 20 nm, reflecting the content of amylopectin was much higher than that of amylose. After wet ball milling, the molecular size distribution profile changed significantly. All wet-ball-milled starch samples showed two distinct peaks in the molecular size distribution, with the major peak moved to a lower R_h value while the intensity of the secondary peak increased significantly. This indicates the degradation of amylopectin chains resulting in small molecule fractions. In other words, wet ball milling reduced the molar mass of amylopectin, the major component of rice starch, while degraded amylopectin could show a size close to that of amylose. Similarly, Huang et al.²⁰ observed an increase in apparent amylose content for cassava and maize starches.

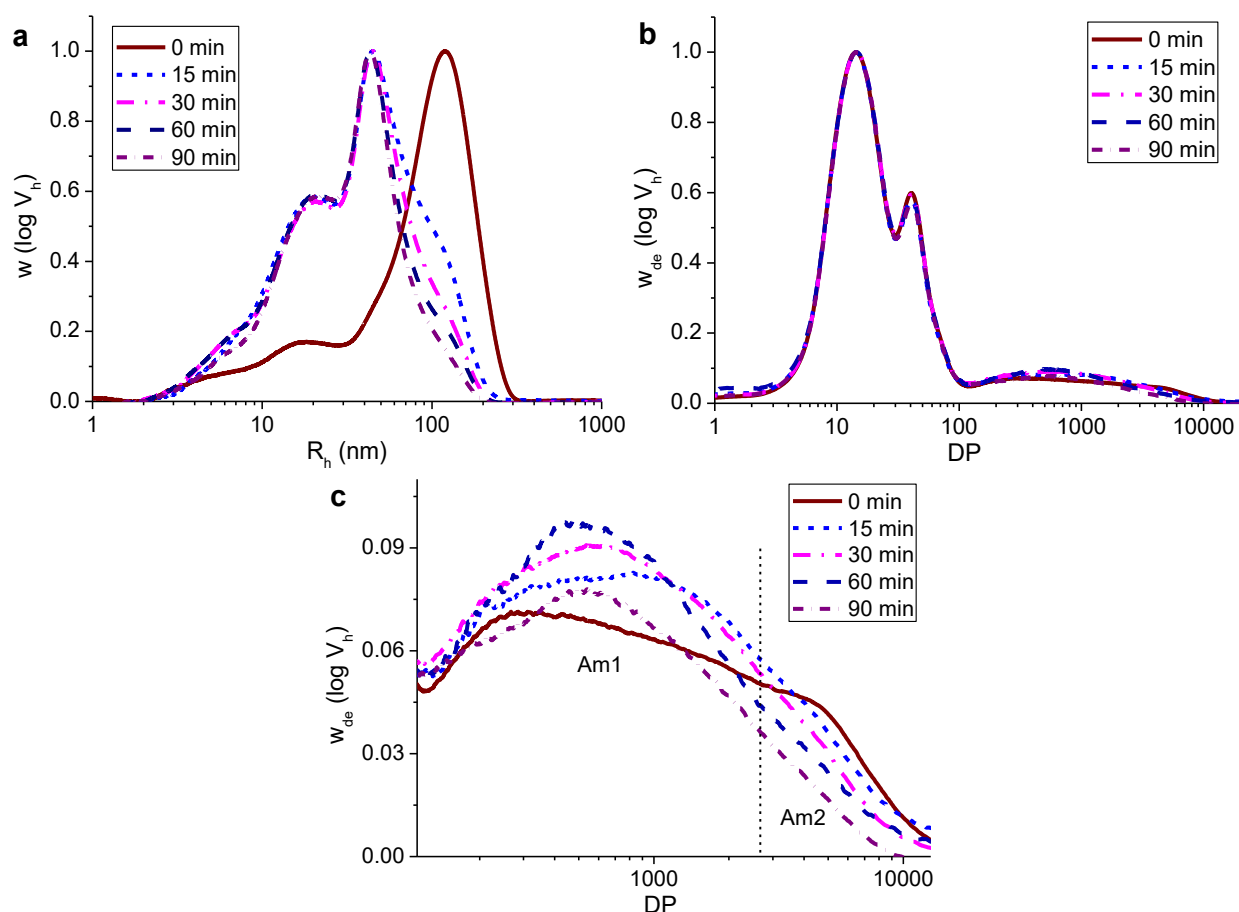


Fig. 6 a) Molecular size distribution of rice starch wet-ball-milled for different durations; b) and c) chain-length distribution of the same starch samples after debranching.

Starch has the advantage that the distribution of degree of polymerization of individual branches can be readily obtained using a debranching enzyme.³⁵ **Fig. 6b** shows that all wet-ball-milled rice starch samples (0–90 min) after debranching displayed no difference in the molecular size distribution profile in the range lower than 100 DP. In this regard, the effect of mechanical force induced by wet ball milling did not cause an apparent change to starch branched chains. However, the curve height changes shown in **Fig. 6c** suggest that, during wet ball milling up to 60 min, amylose or starch backbone chains with a high DP ($> \sim 2600$) (termed as the Am2 fraction) had some degradation which resulted in an increased quantity of those with lower DP (between 100 and ~ 2600) (termed as the Am1 fraction). For the 90-min sample, the curve height in the range over 100 DP was all lower than that for the other samples. With regard to this, likely, a long period of wet ball milling

could significantly degrade amylose or starch backbone chains, resulting in chain fragments with even lower DP (< 100).

3.6 Pasting properties

Fig. 7 shows the RVA curves for rice starch wet-ball-milled for different durations, and the related parameters are listed in **Table 4**. The 0-min sample (without ball milling) showed a well-defined RVA profile with a peak viscosity of 448 cP and a strong setback. Only subjected to 15 min of wet ball milling, the rice starch displayed significantly decreased pasting temperature and overall viscosity, with the peak viscosity being only 24 cP. Longer duration of wet ball milling even decreased the overall viscosity further. It was reported previously that ball milling decreased gelatinization temperature and pasting viscosity for other starches).¹⁹⁻²⁰ This changes in pasting properties for rice starch here could be well correlated with the destruction to the granule, crystalline and lamellar structures and the breakage of amylopectin chains induced by wet ball milling. As a consequence, the mechanically treated rice samples displayed weaker resistance to the swelling and rupture while being heated in water, resulting in reduced pasting temperature and viscosity.

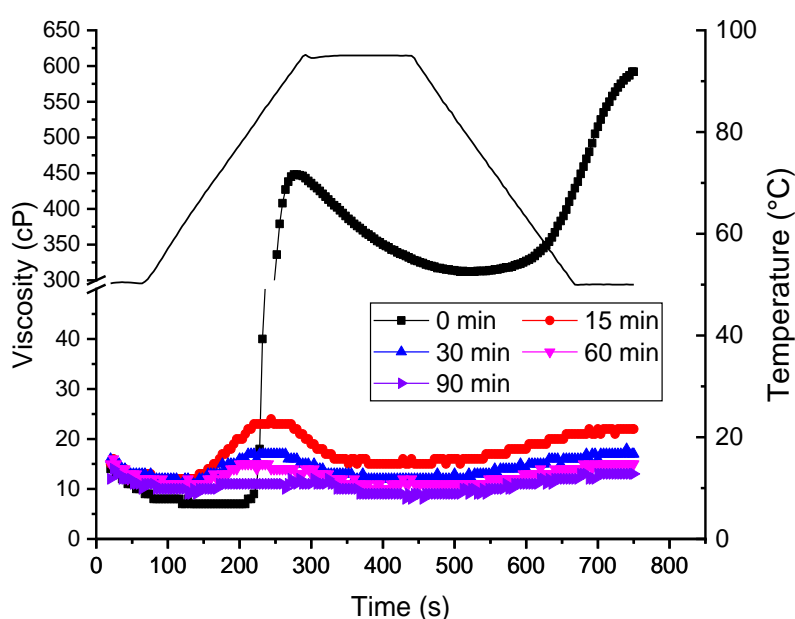


Fig.7 RVA profiles for rice starch wet-ball-milled for different durations.

Table 4 RVA parameters of rice starch wet-ball-milled for different durations.

Ball milling duration (min)	Pasting temperature (°C)	Peak viscosity (cP)	Final viscosity (cp)	Breakdown value (cP)	Setback value (cP)
0	83.9	448	592	136	144
15	—	24	22	9	−2
30	—	17	17	5	0
60	—	15	15	5	0
90	—	12	13	4	1

In conclusion, this work indicates that wet ball milling could be a highly effective physical method to modify the multilevel structures (i.e. granule, lamellar, crystalline, short-range order, and molecular) and pasting properties of *indica* rice starch. We found that only 15 min of wet ball milling caused significant changes to the starch structures and pasting properties whereas changes resulting from longer duration of the treatment were relatively small. The hydromechanical effect provided by wet ball milling could effectively reduce the size of starch granules and destroy lamellar and crystalline structures. The NMR analysis indicates that with prolonged duration of wet ball milling, the proportion of double helices decreased and that of amorphous starch increased, while that of single helices increased moderately. Molecular size distribution results suggest that wet ball milling dominantly degraded long amylose and starch backbone chains but did cause apparent change to starch branched chains. These structural changes can be well correlated with the reduced pasting temperature and viscosity of wet-ball-milled rice starch. The high efficiency of wet ball milling highlights the effect of water in mechanical treatment (in analogy to the effect of water in thermal

treatment) on starch multilevel structures. The knowledge obtained from this work could be useful for designing cost-effective mechanical processes for rice starch-based foods with tailored structure and physicochemical properties.

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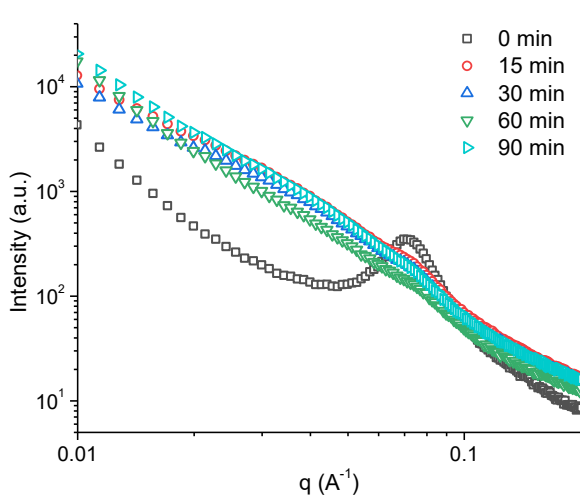
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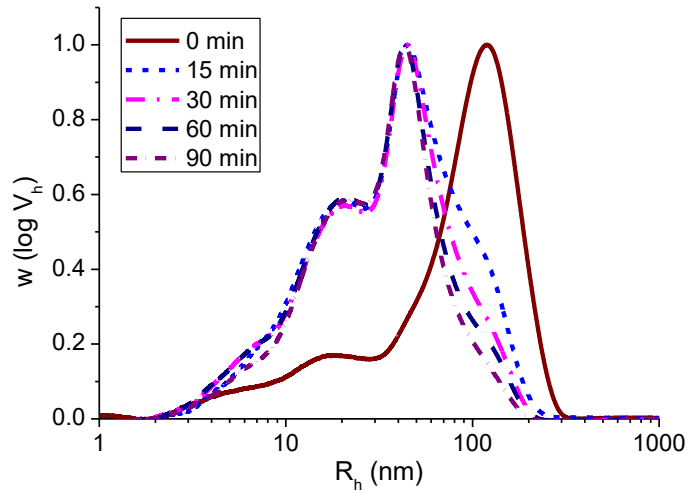
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Table of Content Graphic



SAXS showing lamellar structure



Molecular size distribution

Wet ball milling of *Indica* rice starch: the most effective changes to multilevel structures occurred within 15 min; higher single helix content