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Physicochemical and Morphological Analyses on Damaged Rice Starches

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ABSTRACT

Two types of rice cultivars, indica type (Tainung Sen 19, TNuS19) and waxy (Taichung Waxy 70, TCW70) were used as samples to investigate the changes in the physicochemical properties of damaged rice starch by ball-milling treatment. Rapid Visco Analyzer (RVA) analysis indicated that onset temperature ($T_{o,\eta}$), peak viscosity (P), setback (C-H), and cold-paste viscosity (C) decreases were more pronounced in TNuS19 than in TCW70 starch during the ball-milling treatment. Similar phenomena were also found in peak temperature (T_p) and enthalpy changes (ΔH) by differential scanning calorimetry (DSC), and the peak intensities measured by X-ray diffraction. However, both types of starch showed that extended treatment time resulted in higher median particle size (PS 50). Polarized light micrographs showed that damaged starch granules lost their birefringence, suggesting that the order structure of starch granule was disrupted. The morphological changes in the microstructure of rice starch during ball-milling treatment was also examined by scanning electron microscopy (SEM). The results indicated that TNuS19 starch granules may be more susceptible to mechanical action than TCW70 starch.

Key words: rice starch, ball-milling, damaged starch, rice cultivar changes

INTRODUCTION

When polished rice kernels are ground into rice flour, some starch granules are damaged by the mechanical action of the milling process[1-7]. Recently, damaged starch has been a concern for rice flour production in Taiwan. Chen et al.[1] indicated that the damaged starch and particle size distribution are two key factors affecting the physicochemical properties and the applications of rice flour. The damaged fraction of rice flour may be distinguished microscopically by staining a sample with dyes or from the detection of a loss of birefringence[8,9]. Damaged starch swells extensively in cold water, and the swelling is limited to the damaged portion of the starch granules[10]. The rice flour with a high level of damaged starch generally had high water absorption capacity and was more susceptible to attack by amylase[9-11].

During rice flour milling process, mill type and milling method can profoundly affect the physicochemical characteristics of rice flours[1,2]. Generally, wet-milled rice flour is better than dry-milled flour for making traditional rice-based products baked or steamed[2,7,12]. Chen et al.[2] reported that the wet-milled rice flour gave the lowest damaged starch level and the finest particle size among dry- or semi-dry milled flours. Additionally, the degree of damage is also affected by rice kernel hardness, mill types, milling methods and the soaking process[1,13,14].

Although many milling parameters (i.e. rice cultivar, mill type, milling method and soaking process) on the physicochemical characteristic of rice flour have been investigated, the mechanism of mechanical action on rice flour and starch remains unclear. A better understanding of the mechanical effects would be helpful for the manufacturing of rice flour. It was therefore appropriate to study the effects of physical damages on rice starch before considering the more complex effects of the industrial milling process. In a previous study[8], we have described the effects of the ball-milling treatment on various degrees of starch damage and morphology changes of waxy and non-waxy rice starches. The objectives of this study were to investigate the effects of the ball-milling treatment on the physicochemical properties and the structural integrity of rice starches. The mechanical effects on starch pasting, gelatinization, particle size distribution, microscopic appearance and crystallinity properties were examined.

MATERIALS AND METHODS

I. *Rice Starch and Ball-mill Treatment*

Rice starch from two cultivars, Tainung Sen 19 (TNuS19) and Taichung Waxy 70 (TCW70) were used as samples. Starch isolation and the determination of amylose content were described in a previous work[8]. Ball-milling procedures were also the same: 125 g of rice starch was placed with 10 stainless steel balls (16-mm diameter) in the mill (Fritsch, Type: 06.101, Germany). The operation was

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done at 420 rpm and room temperature. The milled samples were collected at 10, 30 and 60 min, respectively. All tests were run in triplicate.

II. *Analytical Methods*

(I) *Rapid visco analyzer (RVA)*

The pasting behavior of the starch sample was determined with a Rapid Visco Analyzer (RVA 3D, Newport Scientific Pty., Ltd., Narrabeen, Australia). A 28 g of suspension containing 3 g of starch was used. The determination was carried out by heating at 50˚C for 1.5 min, raising to 95˚C in 3.7 min, holding at 95˚C for 2 min, followed by cooling to 50˚C in 3.7 min and keeping at 50˚C for 6.1 min[2].

(II) *Differential scanning calorimetry (DSC)*

The sample of starch: water = 1:3 (w/w) was prepared and kept in a refrigerator overnight for reaching equilibrium. 110 ± 10 mg of the above starch suspension was put into a stainless steel crucible, and sealed with a stainless steel cover with an aluminum O-ring. The sealed sample crucible was scanned by a differential scanning calorimeter (Setaram DSC121; Caluire Cedex, France) from 20˚C to 150˚C at 5˚C /min[2].

(III) *Particle size distribution*

The particle size distribution of 0.02% sample suspended in ethanol was measured by a laser particle size analyzer (Fritsch Analysette 22, Germany). The median particle diameter (PS 50 in μm) was calculated using the software provided by Fritsch Co.

(IV) *Microscopy*

Light microscopy and scanning electron microscopy (SEM) were used to examine the starch granules. Samples were observed using a polarized light microscope (Nikon AFT-IIA, Japan)[1]. For SEM, all samples were mounted on aluminum stubs using double-sided tape, sputter-coated with gold and investigated using an ABT-150S SEM (Topon Corp., Japan) at an accelerated voltage of 15 kV.

(V) *Powder X-ray diffraction*

An X-ray diffractometer (D/MAX-IIIA, Rigaku Denki Co. Ltd., Japan) with the method of Zobel[15] was used.

(VI) *Statistical analysis*

Data were analyzed by Statistical Analysis System (SAS)[16]. Analysis of variance (ANOVA), correlation and Duncan's multiple range test were performed when appropriate.

RESULTS AND DISCUSSION

I. *Pasting Behavior*

The rapid viscosity changes of native and ball-milled starch suspensions during RVA measurements are shown in Figure 1. Rice cultivars and ball-milling treatment time affected all pasting parameters of the starch suspensions, including the onset and peak temperatures ($T_{o,\eta}$ and $T_{p,\eta}$ respectively), peak viscosity (P), hot-paste viscosity (H), breakdown (P-H), cold-paste viscosity (C) as well as setback (C-H). The results indicated that both TNuS19 and TCW70 ball-milled starches tend to exhibit lower $T_{o,\eta}$, P, C-H, and C viscosity than native starches, and were more pronounced in TNuS19 starch than in TCW70 starch. TNuS19 starch showed significant decreases in $T_{p,\eta}$, P, C-H and C as treatment time prolonged. However, TCW70 starch showed a slight decrease for 10 min, but significantly decreased for 30 and 60 min of treatment. After 30 min of treatment, both TNuS19 and TCW70 ball-milled samples showed cold water viscosity (Figure 1). The high proportion of damaged starch probably contributed to their high water retention capacity[8]. The statistical analysis showed

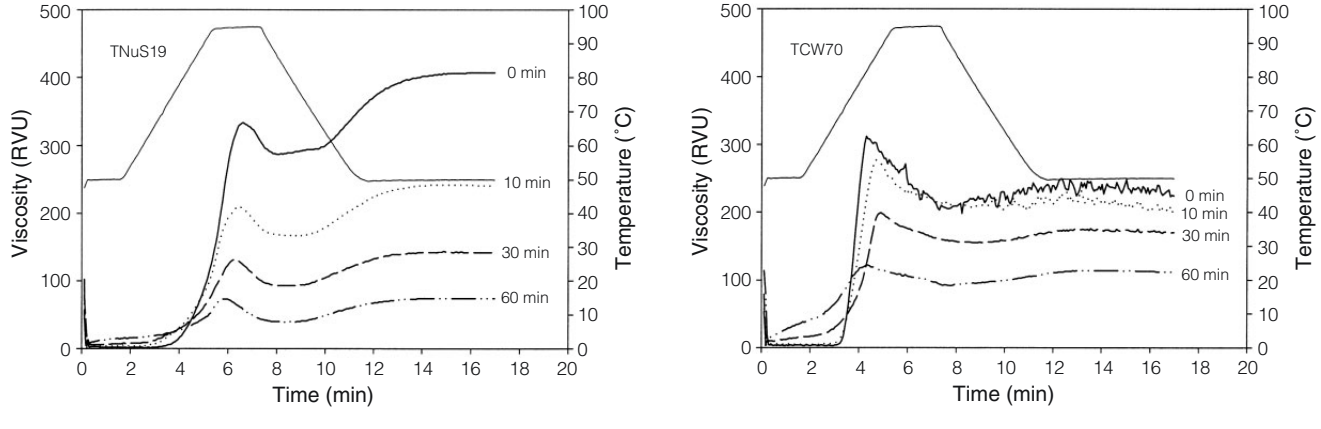

Figure 1. The effect of ball-milling treatment on pasting characteristics of TNuS19 and TCW70 rice starches.

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that ball-milling treatment time was negatively correlated with $T_{o,\eta}$ ($r = -0.91$, $p < 0.01$), P ($r = -0.92$, $p < 0.01$), H ($r = -0.89$, $p < 0.01$) and C ($r = -0.83$, $p < 0.01$) (Table 1). Many researchers have reported that ball-milling caused starch molecules (i.e. amylopectin and amylose) to break down into low molecular weight fragments, which could have caused decreases in the paste viscosity of starches[11, 17, 18, 19]. Morrison and Tester[19] suggested that low molecular weight fragments (LMWAP) of amylopectin were formed by breaking glycosidic bonds in B_2, B_3, and B_4 internal chain segments of amylopectin.

II. *DSC*

Figure 2 and 3 show the effect of ball-milling treatment on the DSC thermograms of TNuS19 and TCW70 starches, respectively. The results indicated that the TNuS19 sample significantly ($p < 0.05$) decreased the onset (T_o), peak (T_p), and conclusion (T_c) temperatures as well as enthalpy changes (ΔH) after the ball-milling treatment. However, TCW70 showed slight change for 10 min, and significantly decreased ΔH for 30 and 60 min treatment, which indicated the order structure of the starch granule was disrupted. The statistical analysis showed that ΔH was negatively correlated with ball-milling treatment time ($r = -0.84$, $p < 0.01$), and positively correlated with pasting parameters $T_{o,\eta}$ ($r = 0.73$, $p < 0.05$), P ($r = 0.95$, $p < 0.01$), P-H ($r = 0.87$, $p < 0.01$), H ($r = 0.85$, $p < 0.01$) (Table 1). The results indicated that the effects of ball-milling may be attributed partly to the conversion of starch into a more amorphous form as treatment time increased. Morrison et al.[20] suggested the decreases in T_o, T_p, T_c and ΔH with increasing level of damaged starch for wheat and maize starch. Several studies have reported that amylose in normal starch was much less affected by physical impact compared with amylopectin[17,18,19]. Tester et al.[21] observed that small B-granules of wheat starch were more susceptible to damage than large A-granules. Mok and Dick[22] indicated that isolated starch from hard wheat is more susceptible to physical action than that from soft wheat. From our previous study[8], we have shown that increasing ball-milling periods caused a higher degree of starch damage ($p < 0.05$), and TNuS19 starch were more susceptible to damage than TCW70. Lu et al.[23] reported that the amylopectin of indica rice had lower molecular weight, lower average degree of polymerization (DP), and lower average chain number when compare to japonica and

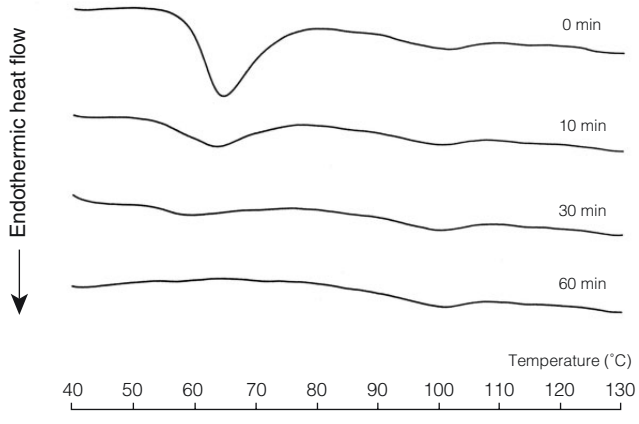

Figure 2. The effect of ball-milling treatment on DSC therograms of TNuS19 rice starch.

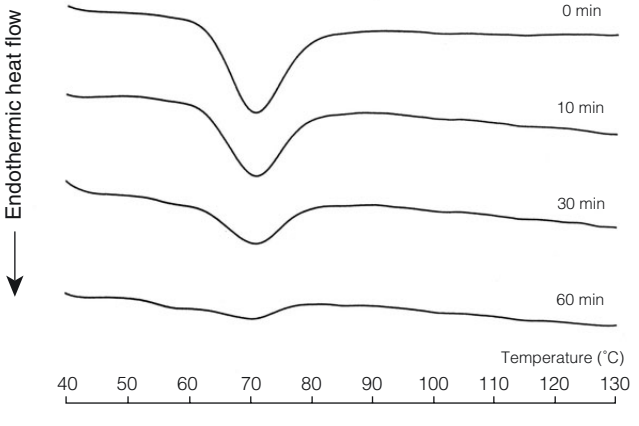

Figure 3. The effect of ball-milling treatment on DSC therograms of TCW70 rice starch.

Table 1. Correlation matrix between ball-milling time, pasting behaviors[a] and thermal characteristics[b] of ball-milled rice starches

	Treatment time	$T_{o,\eta}$	P	P-H	C-H	H	C	T_o	T_p	T_c
$T_{o,\eta}$	-0.91**[c]									
P	-0.92**	0.81*								
P-H	-0.66	0.57	0.70							
C-H	-0.54	0.67	0.48	-0.07						
H	-0.89**	0.79*	0.97**	0.51	0.59					
C	-0.83**	0.81*	0.87**	0.31	0.82*	0.94**				
T_o	-0.55	0.41	0.61	0.35	0.06	0.61	0.46			
T_p	-0.55	0.40	0.60	0.35	0.04	0.60	0.45	1.00**		
T_c	-0.57	0.43	0.60	0.35	0.07	0.61	0.47	1.00**	1.00**	
ΔH (mJ/mg)	-0.84**	0.73*	0.95**	0.87**	0.25	0.85**	0.69	0.55	0.54	0.53

[a]All pasting parameters of the starch suspensions, including the onset temperatures ($T_{o,\eta}$), peak viscosity (P), hot-paste viscosity (H), breakdown (P-H), cold-paste viscosity (C) as well as setback (C-H).

[b]Thermal parameters: T_o: onset temperature; T_p: peak temperature; T_c: conclusion temperature; ΔH: enthalpy.

[c]One asterisk indicates signifcance at the 0.05 level; two asterisks indicate significance at the 0.01 level.

waxy for 14 Taiwan rice cultivars. It was suggested that the mechanical effects on pasting and gelatinization properties were more pronounced in TNuS19 starch than TCW70 starch granules and may differ with respect to compositional or granule structural differences between these two starches.

III. *Particle Size Distribution*

The particle size distribution of native and ball-milled starches were determined by laser particle size analyzer (Figure 4). The data showed that the particle size in TNuS19 starch significantly increased after 10 min of treatment, but those in TCW70 did not. After 30 min of treatment, the median particle diameter (PS 50 in μm) of TNuS19 increased from 6.2 μm to 12.1 μm, but those of TCW70 (6.2 μm) did not change. Both rice varieties showed a significant increase in PS 50 after 30 and 60 min of treatment. PS 50 increased with increasing ball-milling time and was probably due to some stacked starch granules. Meuser et al.[17] reported that ball-milling increased the number of free hydroxy groups to form hydrogen bonding of starch molecules.

IV. *Microscopic Examination*

Polarized light micrographs of native and damaged TNuS19 starch are shown in Figure 5. The result indicated that TNuS19 starch granules significantly lost their birefringence as treatment time prolonged, suggesting that the ordered structure of the starch granule was disrupted. Similar phenomena were also found in TCW70 starch (data not shown). From our previously study[8], iodine and congo red staining analysis showed that the morphology of

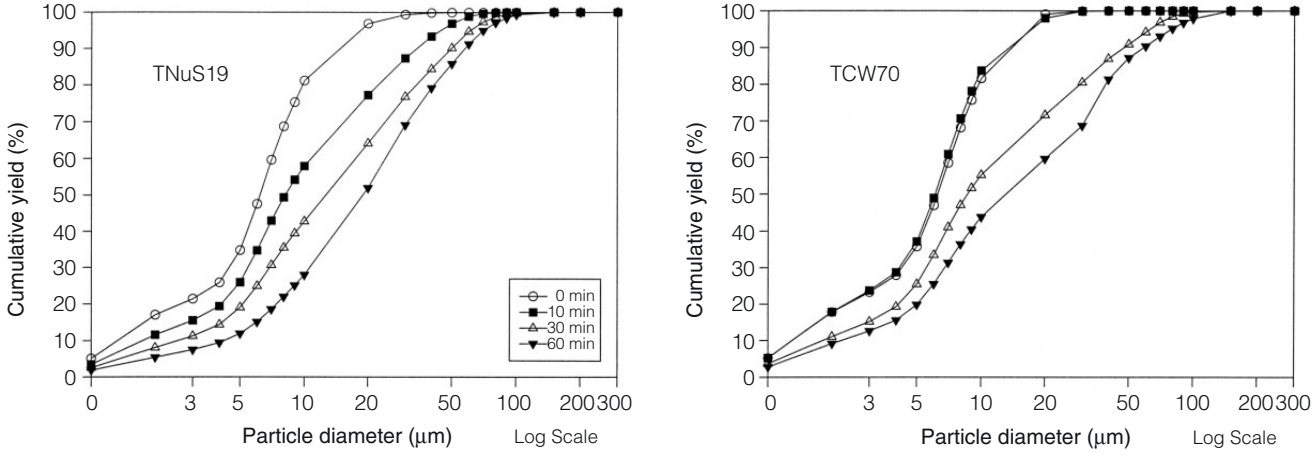

Figure 4. The effect of ball-milling treatment on particle size distributions of TNuS19 and TCW70 rice starches.

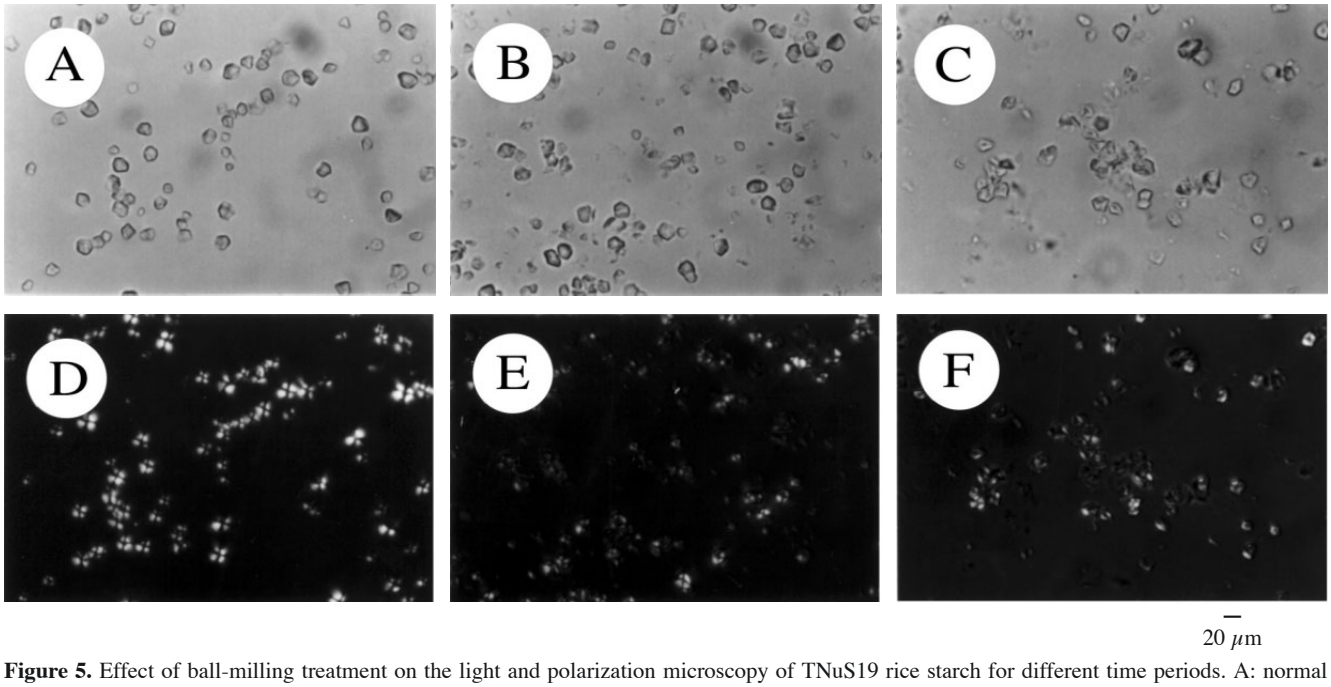

Figure 5. Effect of ball-milling treatment on the light and polarization microscopy of TNuS19 rice starch for different time periods. A: normal light, 0 min; B: normal light, 10 min; C: normal light, 30 min; D: polarized light, 0 min; E: polarized light, 10 min; F: polarized light, 30 min.

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damaged TNuS19 starch was very different to TCW70 starch. Damaged TNuS19 starch gave a more granular form, while TCW70 starch gave more random disrupted shapes after the absorption of water. This result may be due to amylose molecules that can hold the starch molecules together[8].

SEM clearly indicated that the surfaces of the starch granules lost flatness and smoothness, and became rough as milling time continued (Figure 6). Both rice cultivars significantly showed that some granules had attached to each other after 60 min of treatment (Figure 7). These results suggest that glycosidic bonds were broken during ball-

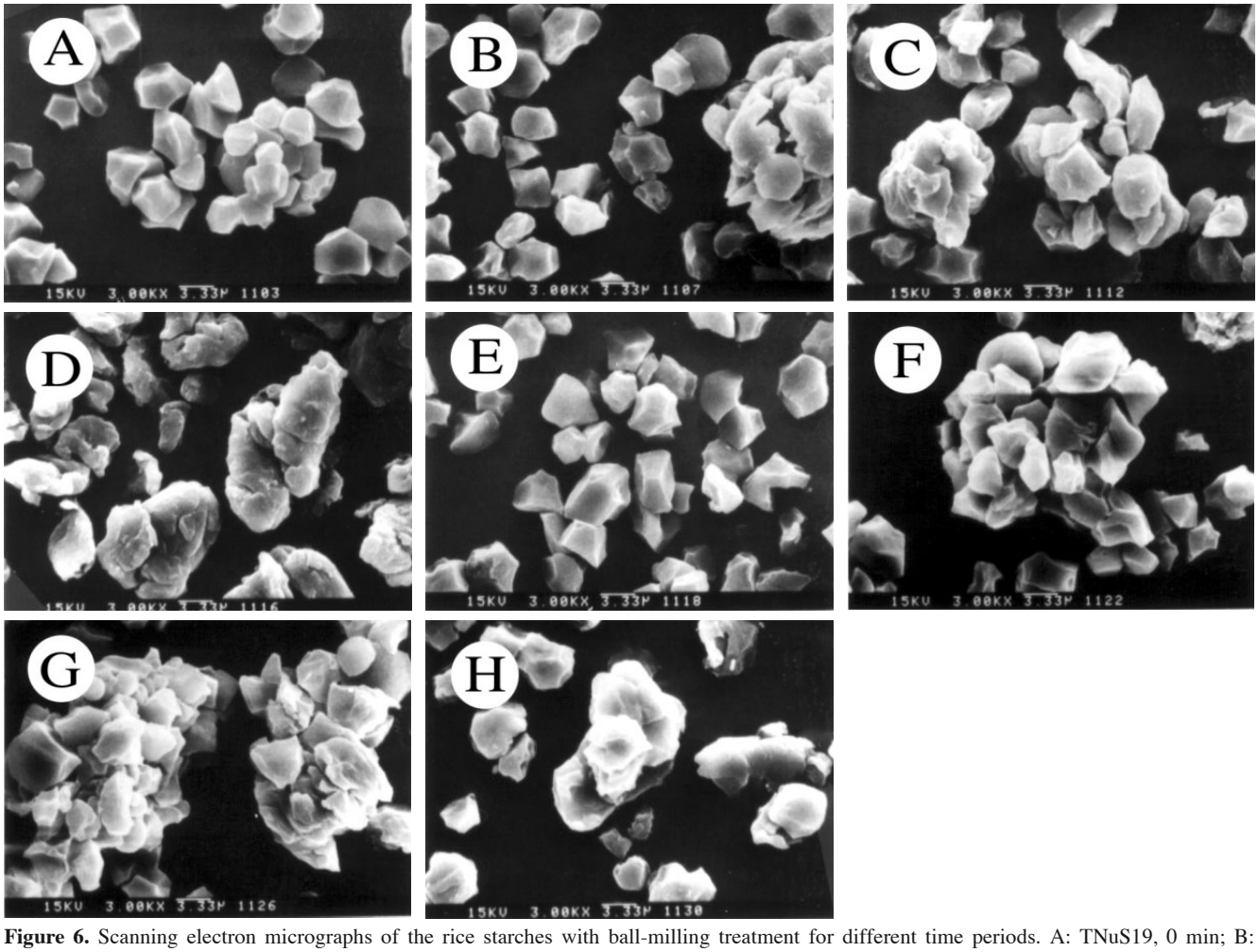

Figure 6. Scanning electron micrographs of the rice starches with ball-milling treatment for different time periods. A: TNuS19, 0 min; B: TNuS19, 10 min; C: TNuS19, 30 min; D: TNuS19, 60 min; E: TCW70, 0 min; F: TCW70, 10 min; G: TCW70, 30 min; H: TCW70, 60 min.

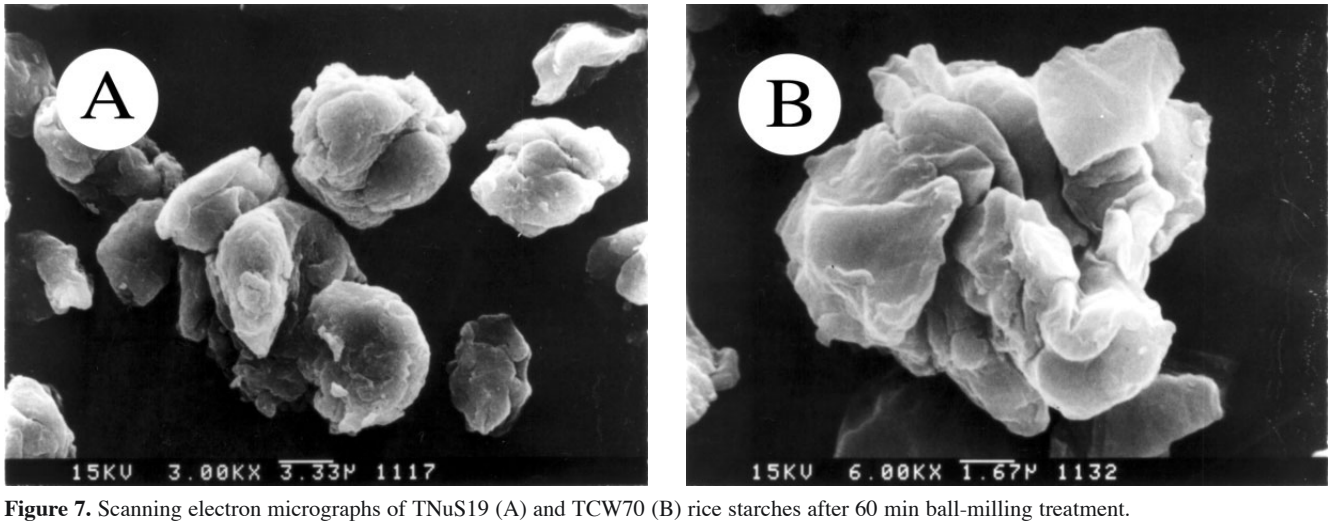

Figure 7. Scanning electron micrographs of TNuS19 (A) and TCW70 (B) rice starches after 60 min ball-milling treatment.

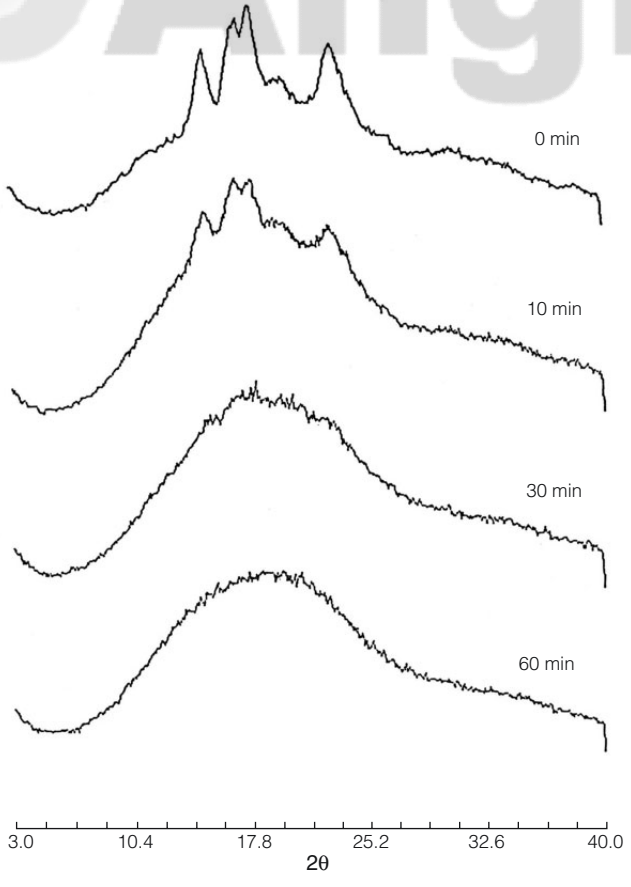

Figure 8. Effect of ball-milling treatment on X-ray diffraction patterns of TNuS19 rice starch.

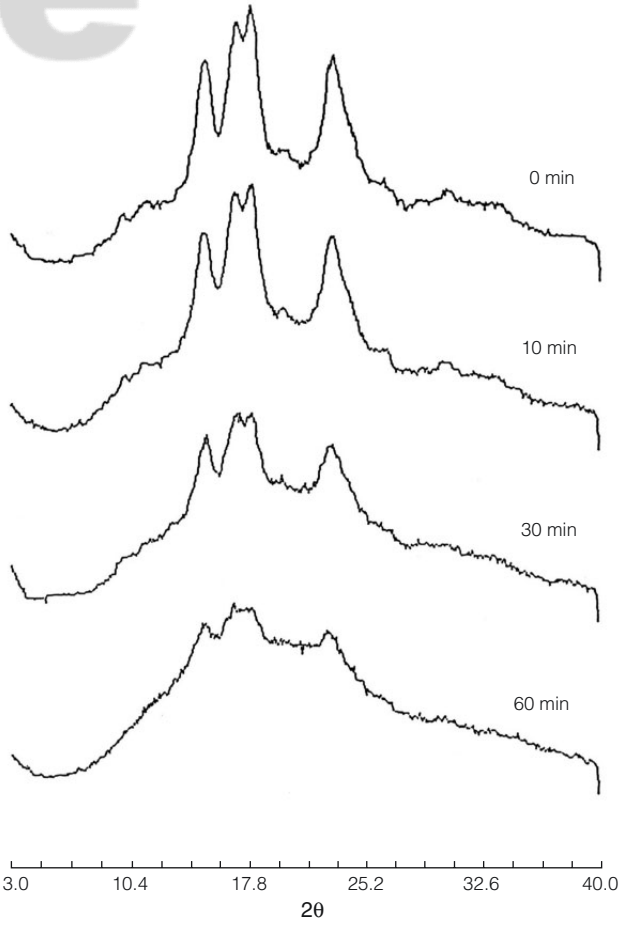

Figure 9. Effect of ball-milling treatment on X-ray diffraction patterns of TCW70 rice starch.

milling and the result increased free hydroxy groups to form hydrogen bonding between starch molecules. These results confirmed the findings of a previous researcher[17]. The surface of the granules was rough and cracks were also found in corn and barley starch after ball-milling[11, 21].

V. *X-ray Diffraction*

The effects of ball-milling treatment on X-ray diffraction properties of TNuS19 and TCW70 starches are shown in Figure 8 and 9. The results of X-ray diffraction indicated that both starches showed A type pattern, and ball-milled samples showed significant decrease in X-ray peak intensities. TNuS19 showed significant decreases in peak intensities after 10 min of treatment, but TCW70 did not. TNuS19 starch almost lost peak intensity after 30 min and completely lost it after 60 min. However, TCW70 starch retained the pattern through 60 min of ball-milling treatment. The results indicated that the effects of milling may be attributed partly to the conversion of starch into a more amorphous form as treatment time increased. Morrison and Tester[19] reported that amylose is barely affected by physical damage to the granule, but amylopectin is progressively degraded into low-molecular-weight fragments. A ^{13}C solid-state-NMR spectra of wheat starches suggests that disruption occurs at the glycosidic linkage during ball-milling and resulted in increases in free hydroxyl groups and the decomposition of polymers[24].

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