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The effects of ball mill processing on the physicochemical properties of glutinous rice starch

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ABSTRACT

Glutinous rice starch (GRS) (*Oryza sativa* L.) is a product derived from a staple food that is commonly cultivated and consumed in Asia. This study investigates the effect of physical modification by planetary ball mill processing on some physicochemical properties of GRS to increase the value of GRS for further pharmaceutical application. Design of experiment: two-factor-three-level factorial design was utilized to design and interpret the results in this experiment. Speed (X_1) (100-400 rpm) and time (X_2) (30-120 minutes) of milling were two factors examined in this study. The relationship between the two parameters allowed individual examination of four properties; the swelling capacity (SwC) (0.71±0.037-3.42±0.084), percent cold water solubility (%CWS) (0.533±0.047%-26.708±1.598%), percent gelatinization (1.178±0.606%-25.314±0.957%), and particle size (47.131±2.899 μm-306.286±2.031 μm). Increasing the milling speed increased all four properties, whereas increasing the milling time only increased %CWS and particle size. The investigation demonstrated a positive effect on %CWS from the interaction of milling speed and time (X_1X_2). The quadratic of milling speed (X_1^2) negatively affected SwC, while particle size was affected in a positive way. X-ray diffractogram and surface morphology analysis substantiated the milling response data.

Keywords: Ball mill, design of experiment, factorial design, glutinous rice starch, *Oryza sativa* L., physicochemical properties

INTRODUCTION

Glutinous rice, waxy rice, or sticky rice (*Oryza sativa* L.) is a staple food which is commonly cultivated and consumed in Asia.[1] Rice starch produced from broken rice grains can be processed to reduce protein content.[2] Starch is generally comprised two types of glucose biopolymers; linear amylose and branched amylopectin linked by α-1,4 and/or α-1,6 glycosidic bonds.[1] Amylose content in glutinous rice starch (GRS) is normally <2%. Therefore, amylopectin is a component of major importance in the physicochemical characteristics of GRS.[1,3] GRS has long been widely used in the food industry. However, only a few studies on pharmaceutical usage are available. This is due to instability associated with varying temperature, shear, and pH condition.[4-6] Modified GRS is one option for product development in pharmaceutical industrial applications.

Planetary ball mill is a physical modification which applies the friction, collision, impingement, shear, or other mechanical actions to modify the structure and properties of starch granule.[7,8] The process is simple, inexpensive, chemical free, and eco-friendly. However, it produces irregularly shaped particles.[9] Processing set up is needed to control the specific energy when transferring from the laboratory scale to the manufacturing scale.[10] Ball mill is popularly used for various kinds of starch modification such as corn starch,[7,11,12] cassava starch,[8,13] jicama starch,[13] wheat starch,[14] and rice flour,[15,16] among others. Interestingly, there is no published information available on the effect of ball mill processing for the physicochemical properties of GRS.

The previous studies suggest that different ball milling parameters can provide some changes in physicochemical properties. Product transformation can be manipulated for controlled results by varying milling speed, milling time, moisture content, pre-treatment process, type of ball mill pot, and other processing variables.[7,8,13,15,16]

In this study, GRS was physically modified with a planetary ball mill at various speeds and time durations. A two-factor-three-level-factorial design was selected for this research. Six responses were studied; swelling capacity (SwC), cold water solubility (CWS), percent gelatinization, particle size, surface morphology, and crystallinity. Four obtained mathematical models were observed and statistically compared. The effect of each factor on each response was studied.

MATERIALS AND METHODS

Materials

Native GRS (nGRS) from the Thai Flour Industry (Bangkok, Thailand) was used in this study. Its composition is nearly 100% amylopectin. The chemicals and/or reagents used in the experiments were of analytical grade.

The Experimental Design for Preparation of Ball Milled GRS (BM-GRS)

The two-factor-three-level-factorial design was used for the experimental plan. Two critical factors were manipulated during testing, including speed (X_1) and time (X_2) of ball milling. The range of both factors was selected according to the capability of the ball mill apparatus. Speed of milling was studied in the range of 100–400 rpm and milling duration was studied in the range of 30–120 min. The minimal, central, and maximal values represent as coded level −1, 0, and 1, respectively. An experimental test of 9 runs was initiated. Two replications at the center point were added, resulting in a total of 11 runs (Design-Expert® software, version 9.0, Stat-Ease, Minneapolis, MN), as shown in Table 1.

Planetary ball mill (PM100 Retsch®, Germany) equipped with zirconium oxide jar and balls were used in this experiment. This equipment can be utilized for hard or abrasive materials, long-term trials, and heavy-metal-free grinding without material color change.[17] A preliminary study was performed to determine the appropriate amount of material and number of balls that provided the highest yield without sample attaching to the lid. The 250 ml jar was filled with 70 g (one-third of the capacity) of GRS, 3 balls (3 cm diameter), and rotated horizontally without rotational direction change. Six physicochemical properties of BM-GRS were evaluated. Four responses, which were included in DoE analysis, were the SwC (SwC, Y_1), CWS (CWS, Y_2), percent gelatinization (Y_3), and particle size (Y_4). The physicochemical data were analyzed by Design-Expert® software and mathematical models were obtained. The model would be considered acceptable if it met the requirements, i.e., the model *P*-value was significant ($P < 0.05$), lack of fit *P*-value was non-significant ($P > 0.05$), and R-square value was higher than 0.7, indicating a high correlation between the factors and the responses.[18] The X-ray diffraction (XRD) (Y_5) and scanning electron microscope (SEM) analysis (Y_6) were utilized as supportive data.

Evaluation of Physicochemical Properties of GRS

SwC

The GRS (4 g) was weighed in 25 ml cylinder with a stopper. The initial volume was determined as V_1. Then, 20 ml of distilled water was added and shaken until all the particles were well dispersed (around 5 min). The starch dispersion was adjusted to 25 ml with distilled water and left to completely swell, taking 24 h at room temperature. The final volume of GRS was determined as V_2. The SwC was calculated using equation 1.[19]

$$SwC = V_2/V_1 \quad (1)$$

CWS

The GRS (0.75 g) was weighed in 50 ml centrifuge tube, adjusted to 15 ml volume using distilled water. It was then left for a well-dispersed for 30 min at room temperature.

Table 1: The condition of ball milling is shown in both actual values and coded levels for each run of two-factor-three-level-factorial design and the physicochemical properties of native glutinous rice starch and ball milled glutinous rice starch

Runs	Factors		Responses			
	X_1 (speed) (rpm) actual (code)	X_2 (duration) (min) actual (code)	Y_1 (swelling capacity) (*n*=3)	Y_2 (cold water solubility) (%) (*n*=3)	Y_3 (gelatinization) (%) (*n*=3)	Y4 (particle size) (μm) (*n*=3)
Native glutinous rice starch	-	-	0.80±0.037	0.484±0.019	-	49.684±2.883
1	100 (−1)	75 (0)	0.80±0.029	0.755±0.020	1.178±0.606	48.286±2.942
2	250 (0)	30 (−1)	2.49±0.120	4.320±0.334	12.913±1.934	66.739±1.835
3	100 (−1)	30 (−1)	0.86±0.027	0.533±0.047	3.119±0.788	47.131±2.899
4	250 (0)	120 (1)	3.39±0.089	11.452±0.963	14.656±0994	101.791±2.091
5	400 (1)	75 (0)	1.48±0.052	14.750±1.386	23.364±1.381	229.054±1.933
6	250 (0)	75 (0)	3.42±0.084	7.724±1.062	9.15±1.010	96.468±3.922
7	100 (−1)	120 (1)	0.71±0.037	0.960±0.020	10.115±2.833	47.306±2.911
8	250 (0)	75 (0)	3.32±0.228	8.209±1.045	7.224±1.241	101.326±1.603
9	400 (1)	30 (−1)	1.39±0.189	7.537±1.648	11.966±0.667	226.567±1.107
10	400 (1)	120 (1)	1.49±0.101	26.708±1.598	25.314±0.957	306.286±2.031
11	250 (0)	75 (0)	3.28±0.079	8.240±1.721	6.406±1.997	121.221±2.499

Each tube was centrifuged (Kubota, Model 6200, Japan) with a speed of 4500 rpm at room temperature (25°C) for 30 min. The supernatant was collected and heated at 110°C until a constant weight was obtained. The final weight was determined. The study was performed in triplicate and the %CWS was calculated using equation 2, where W refers to the solid content of the supernatant and W_0 refers to the initial weight of the starch sample.[20]

$$\%CWS=W/W_0\times100 \quad (2)$$

Percent gelatinization by differential scanning calorimeter (DSC)

The percent gelatinization of GRS was measured using DSC (DSC214 Polyma, NETZSCH®, Germany). Three milligrams of GRS were placed into 40 µl aluminum crucibles and sealed. The sample was heated from 25°C to 250°C at the constant heating rate of 10°C min^{-1}. A sealed empty crucible was used as a reference. Onset temperature (T_0), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH; J g^{-1}) were determined. Enthalpy was calculated by integrating the area under the peak. Percent of gelatinization was calculated using equation 3.[13,21]

$$\text{Percent gelatinization}=((\Delta H_{nGRS}-\Delta H_{BM\text{-}GRS})/(\Delta H_{nGRS}))\times100 \quad (3)$$

Particle size and size distribution

GRS particle size and size distribution were determined using a laser diffraction particle size analyzer (Mastersizer 2000 Model Scirocco 2000, Malvern Instrument, UK). The dry cell was used since samples were a dry powder. Five grams of sample was sieved and placed into the sample holder and used for three repeated analyses. Average particle size and size distribution results were recorded.

XRD analysis

The crystallinity of BM-GRS was studied using an X-ray diffractometer (Bruker® D8 Advance, USA). The measurement conditions were X-ray tube – Cu Kα radiation, operated at 40 kV ×40 mA of power, with the wavelength of 1.54 nm continuous scan mode. The sweeping angle was from 5° to 50° with the speed of 4.5° min^{-1}. Percent crystallinity was calculated as a percentage ratio of total crystalline peak area to total diffraction area between 6° and 32°.[22]

Surface morphology

The surface morphology of the powders was observed using a SEM (Hitachi® S-3000N, Japan). GRS samples were mounted on the stubs with double-sided adhesive carbon tape and then coated with gold under vacuum to make the sample conductive. SEM photomicrographs were recorded at ×100.

RESULTS AND DISCUSSION

Physicochemical Characterization of BM-GRS

The BM-GRS from each run appeared as a white powder with different particle sizes. When the GRS was ball milled, the balls were moved in a horizontal direction and were directly in contact with the starch. The starch was pulverized and mixed by repeated high energy impact.[23,24] The ball milling process generates friction between the starch granules. The starch granules interact with the balls and container wall. These forces generate heat which modifies the starch properties.

SwC, Y_1

The SwC of nGRS and several BM-GRS are shown in Table 1. According to Design-Expert® software, the best fit was received for the reduced quadratic mathematical model (P = 0.0001, lack of fit P = 0.0514, R-square = 0.9689). The mathematical equation expresses the relationship of milling speed (X_1) and duration (X_2) to SwC (Y_1) of BM-GRS and is shown in equation 4.

$$Y_1=3.26+0.33\ X_1{}^{*}+0.14\ X_2-2.00\ X_1{}^{2*}-0.20\ X_2{}^{2} \quad (4)$$

Where*=Statistically significant

The equation displayed the contribution of main effects (X_1, X_2) and quadratic terms (X_1^2, X_2^2). The coefficients of each term are related to their effect on the response. Negative coefficients represent antagonistic effects, while positive coefficients represent the synergistic effect of the factors.[25] However, the coefficient in quadratic terms also indicates how the curve is bending. The negative coefficient means the curve will appear in concave form (the apex is at the top), while the positive coefficient indicates a convex form (the apex is at the bottom). A factor with a $P < 0.05$ is considered as having an influence on the response. Moreover, the y-intercept expresses the average of all responses.[26]

It was found that only speed (X_1) and quadratic term of speed (X_1^2) had a significant effect on SwC (P = 0.0198 and <0.0001, respectively). In contrast, milling time (X_2) and quadratic term of time (X_2^2) had no significant influence on SwC (P = 0.2271 and 0.2549, respectively). A positive coefficient of speed (X_1) indicated that an increase of speed would lead to an increase SwC [Figure 1a]. For the quadratic effect of speed (X_1^2), the negative coefficients indicate a concave aspect, which means that after reaching a certain speed, SwC would begin to decrease.

The maximum SwC was obtained when the speed was set at medium level then decreased after achieving maximized the speed. This can be explained by the relationship between SwC and particle size.[15] Smaller particle sizes result in more overall surface area which can enhance the water contact to the GRS particles. When milling speed was increased, the particle size would be decreased. However, when the milling speed reaches a velocity equivalent to the medium speed in this research, the mechanical activation property of GRS induces some small particles to agglomerate. Two sizes of particles were detected at 250 rpm BM-GRS [Figures 2 and 3b]. The smaller-sized BM-GRS granules at 250 rpm had a larger surface area in contact with water molecules, resulting in a higher SwC. In contrast, bigger sized granules were observed at 400 rpm [Figure 3c]. The larger particles or aggregated granules could not be hydrated or expanded as rapidly as the smaller particles, leading to the reduction of SwC when the milling speed was increased. The SwC of BM-GRS was approximately 1–5 times higher than the nGRS. Therefore, BM-GRS could be applied in pharmaceutical formulations as a swelling agent for controlling drug release, as a disintegrant, or as another swellable device. However, it will be necessary to optimize

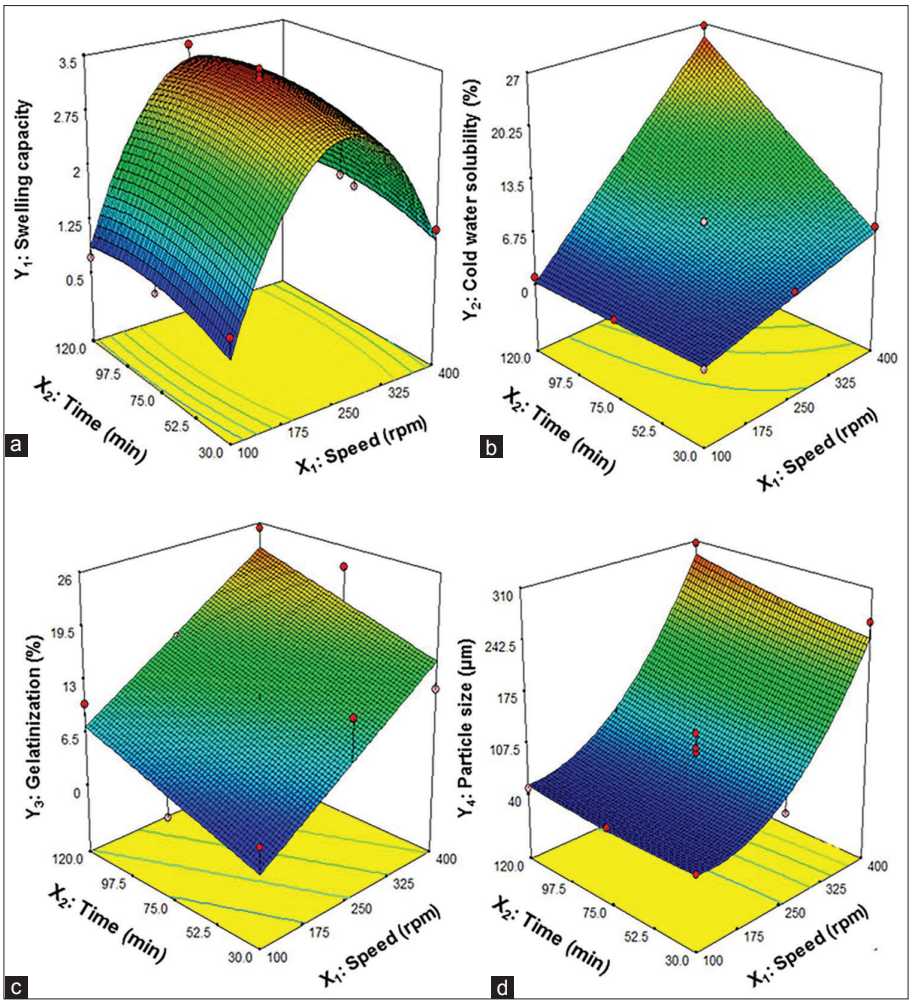

Figure 1: The response surface graph of (a) swelling capacity, (b) % cold water solubility, (c) %gelatinization, and (d) particle size

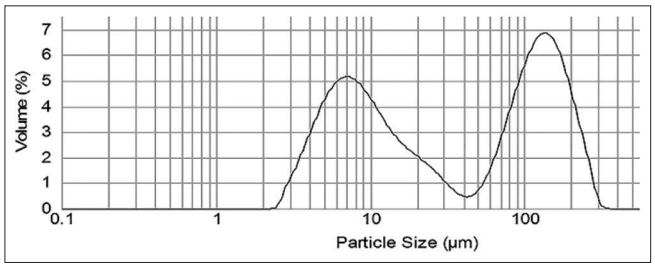

Figure 2: The particle size distribution of 250 rpm/75min ball milled glutinous rice starch

the conditions to generate the appropriate level of SwC as desired.[27]

CWS, Y_2

The CWS of nGRS and various BM-GRS are shown in Table 1. In accordance with Design-Expert software, the best fit was the 2FI (2 factor interaction) mathematical model ($P < 0.0001$, lack of fit $P = 0.0575$, R-square = 0.9876). The mathematical equation for estimating CWS from ANOVA was shown in Equation 5.

$$Y_2=8.29+7.79\ X_1^*+4.46\ X_2^*+4.69\ X_1\ X_2^* \quad (5)$$

Equation 5 represents the relationship between CWS (%) and the determining factors. The results show that speed (X_1), time (X_2), and the interaction term (X_1X_2) had significant positive effects on %CWS (all $P < 0.0001$). When speed and time were increased, %CWS was also increased [Figure 1b]. In addition, and significantly for the interaction term (X_1X_2) from equation 5, the effect of speed on the %CWS was different for different durations of milling [Figure 4]. With shorter milling time (30 min), the % CWS was slightly increased when the milling speed was increased. However, with a longer milling time (120 min), the % CWS dramatically increased when the milling speed was increased.

When starch granules were ball milled, the mechanical damage of the starch would occur and induce a progressive loss of crystalline order.[14] The conversion of ordered crystallinity into disordered amorphous material increases accessibility to external agents such as water. The rigid structure of the crystalline form can prevent water penetration into the starch molecule.[28] A short milling time (30 min) was not long enough for the balls to make sufficient damage to the starch granules to increase the %CWS. Increasing the milling speed gradually increased the %CWS. When employing a long milling time, the results indicate that speed significantly increases the %CWS. In general, nGRS has low solubility in cold water. By increasing

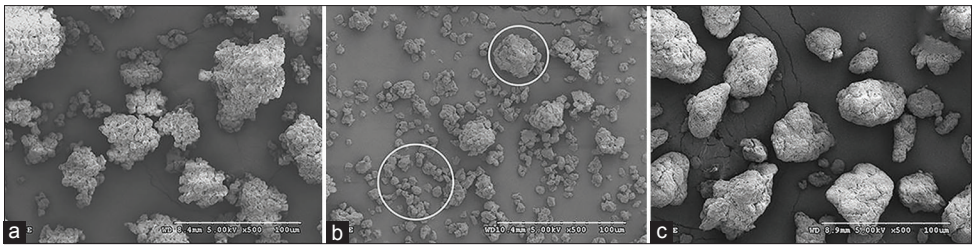

Figure 3: Particle size and shape of (a) native glutinous rice starch (GRS), (b) 250 rpm/75 min ball milled GRS (BM-GRS), and (c) 400 rpm/75 min BM-GRS at a magnification of ×100

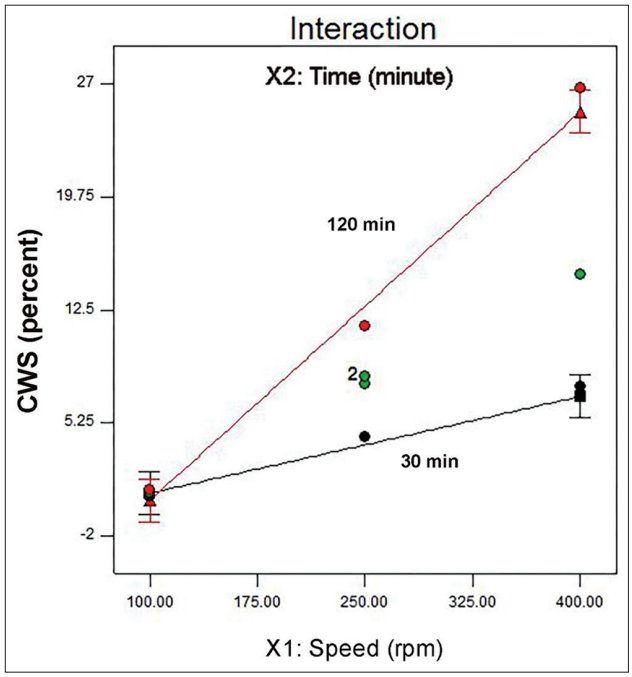

Figure 4: Interaction graph represents the relationship between milling time and speed to cold water solubility response (upper line illustrates the long milling time at 120 min, lower line illustrates the short milling time at 30 min)

its %CWS, BM-GRS can reduce the process of starch paste preparation in drug dosage forms. For example, preparation of starch paste for binder solutions normally requires hot water for dissolving.[29] Therefore, high levels of milling speed and increased time are necessary to achieve the highest %CWS.

Percent gelatinization by DSC

The DSC thermogram of nGRS is shown in Figure 5. There was an endothermic peak at 159.42°C with gelatinization enthalpy of 70.65 J g^{-1}. The percent gelatinization of all BM-GRS is shown in Table 1. According to Design-Expert® software, the best fit was the linear mathematical model (P = 0.0031, lack of fit P = 0.0859, R-square = 0.7648). The mathematical equation estimating the percent gelatinization from ANOVA is shown in equation 6. It represents the relationship between percent gelatinization and the determining factors. Only milling speed had a significant positive effect on the percent gelatinization (P = 0.0018). Time had no significant effect on percent gelatinization (P = 0.0591). When the milling speed was increased, percent gelatinization would also be increased, as shown in Figure 1c.

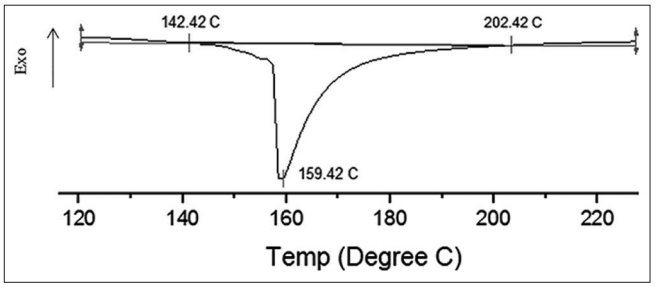

Figure 5: Differential scanning calorimeter thermogram of native glutinous rice starch

$$Y_3=11.40+ 7.71\ X_1^*+ 3.68\ X_2 \quad (6)$$

Gelatinization represents the thermal transition properties of starch.[8] This transition occurs when the presence of heat and moisture leads to structural disruption of the starch molecule. nGRS had 13.17% moisture content. The percent gelatinization is related to the gelatinization enthalpy, which describes the loss of crystallinity and double-helical order.[30,31] The BM-GRS enthalpies were lower than nGRS enthalpy. This relationship indicated that ball milling destroyed the crystallinity and double-helical order arrangements. Severe milling induced slight depolymerization of the amylose chain. Under the same conditions, amylopectin converts to low molecular weight fragments.[13] Increasing milling speed leads to an increasing in the amorphous region. This result is concordant with the result of crystallinity through XRD [Figure 6]. The reduction in crystallinity after high milling speed can induce a higher percent gelatinization. BM-GRS showed some functional characteristics of gelatinization that could potentially be used in food and pharmaceutical systems as a stabilizer, additive, moisture retainer, and thickener.[13]

Particle size

nGRS has the particle size around 49.684 ± 2.883 μm. After ball milling, the particle size and size distribution were measured. D [4,3]-volume weighted mean or the volume of an equivalent sphere was used to represent the particle size of each BM-GRS. The particle size of both nGRS and BM-GRS is shown in Table 1.

According to Design-Expert® software, the best fit was received for the quadratic mathematical model (P = 0.01, lack of fit P = 0.1434, R-square = 0.9800). The mathematical equation for estimating particle size from ANOVA is shown in equation 7.

$$Y_4=95.81+103.20\ X_1^*+19.16\ X_2^*+19.89\ X_1\ X_2+54.74\ X_1^{2*}+0.34\ X_2^2 \quad (7)$$

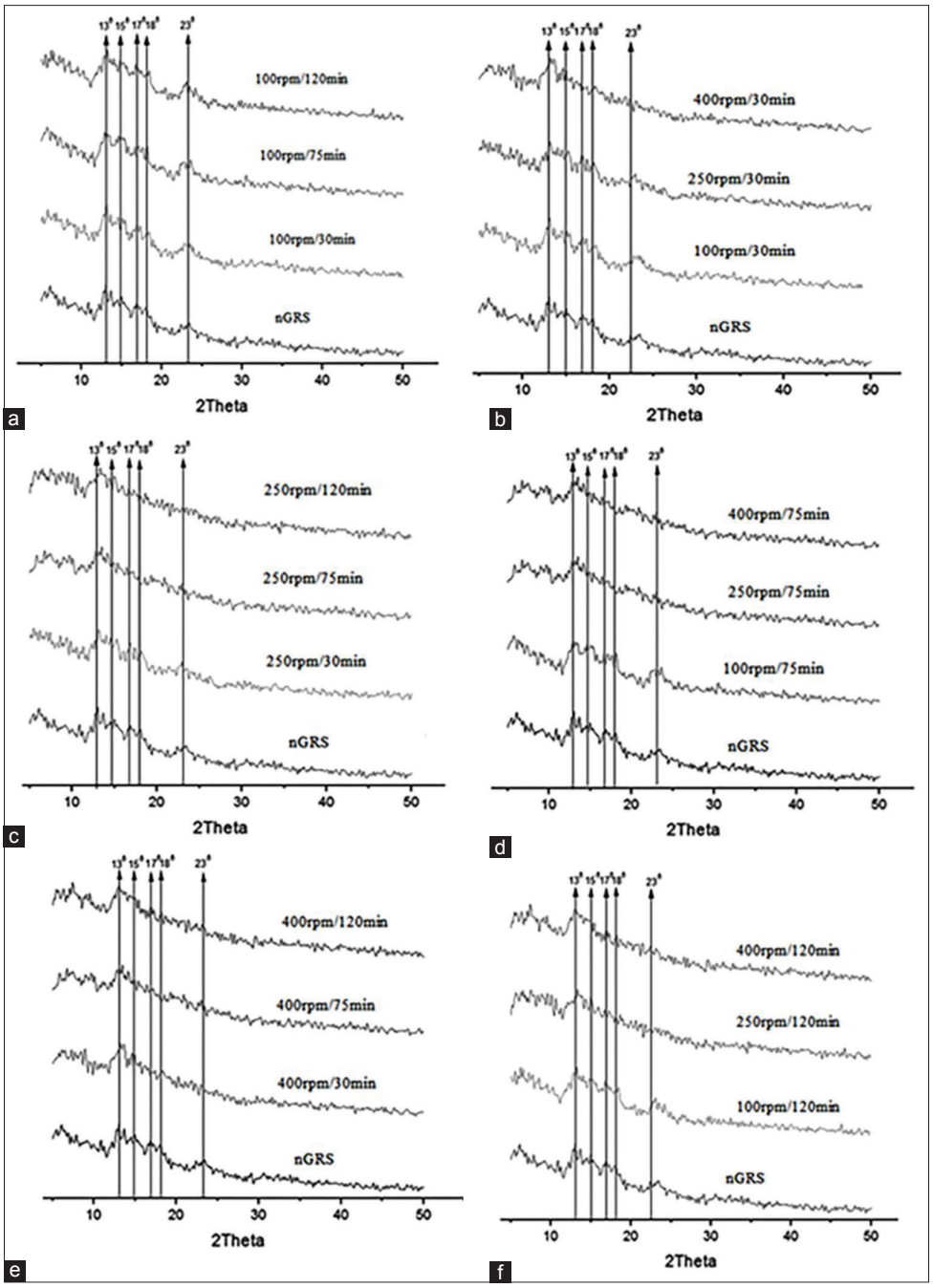

Figure 6: X-ray diffractograms of glutinous rice starch after ball milling in each condition. For (a-c) is a comparison at different milling speed from low to high, respectively, (d-f) is a comparison at different milling time from low to high, respectively

Equation 7 represents the relationship between particle size and the determining factors. Both milling speed (X_1) and time (X_2) demonstrated a pattern of significant positive effects on the particle size ($P < 0.0001$ and 0.0444, respectively) in a positive pattern. When speed (X_1) and time (X_2) were increased, the particle size was also increased. Moreover, the quadratic effect of speed (X_1^2) had positive coefficients which indicated a convex graph (the apex is at the bottom), as shown in Figure 1d. The ball milling process can be divided into grinding and mechanical activation sub-processes. During the milling process, the grinding and mechanical mechanisms are in a dynamic equilibrium that depends on the granule size throughout the tough-brittle transition. During mechanical activation, starch granules are breaking into a smaller size that could clump together into lumps or adhere to the surface of larger granules. The starch granules will be transformed from "brittle" to "tough" state leading to an increase in particle size,[7] as can be seen in Figure 2. At the medium milling time (75 min) and speed (250 rpm), both small and large particle sizes were observed which represented the transition process from grinding (small particle) and lumping (large particle). It can be observed in both Figure 2 and 3b. This confirms that in the quadratic term of speed (X_1^2) with initial milling speed, the milling is first influenced by the grinding process and then by the mechanical activation. This resulted in

Table 2: Summary of the coefficient of the factors for each response

Response	X_1 (Speed)	X_2 (Time)	X_1X_2	X_{12}	X_{22}
Y_1 (swelling capacity)	0.33*	0.14	-	−2.00*	−0.20
Y_2 (%cold water solubility)	7.79*	4.46*	4.69*	-	-
Y_3 (%Gel)	7.71*	3.68	-	-	-
Y_4 (particle size)	103.2*	19.16*	19.89	54.74*	0.34

*Is statistically significant ($P<0.05$)

the smaller particle size at the beginning. After the speed was increased until it reached one certain velocity, the particle size started to increase. Particle size and size distribution are the main factors affecting the flowability and compactibility of tablet excipient formulations, especially direct compression excipients. Coarser excipients provide an advantage for flowability, but fine-sized excipients are better for compatibility.[32] Therefore, these results can help support decisions about conditions to balance flowability and compactibility.

The relationship between each response (Y_1-Y_4) and the studied factors (X_1 and X_2) are summarized in Table 2. It is noticeable that milling speed significantly affected all responses, while milling time only significantly affected %CWS and particle size. There was only one interactional effect from milling speed and time on %CWS. The quadratic term of milling speed (X_1^2) was significantly affected by SwC and particle size.

Surface Morphology

nGRS has a polygonal shape,[6] non-porous, and smooth surface [Figure 3a]. After applying the middle milling speed and time (250 rpm/75 min), the starch particles appeared in both large and small sizes with less edges [Figure 3b]. After the milling speed was increased to maximum velocity (400 rpm/75 min), most of the starch particles appeared in rounded large particles. These results are concordant with the particle size study (3.1.4, Figure 1d, and equation 7).

Crystallinity through X-ray diffractometer

Crystallinity in starch granules has been attributed to amylopectin and is commonly measured by the XRD technique. Based on XRD patterns, rice and other cereal starches (e.g., wheat, barley) are A-type with strong reflections and a crystallinity peak at 2θ 13°, 15°, 17°, 18°, and 23°.[1] The XRD patterns of GRS are shown in Figure 6a-f. The broad and featureless peak reflects an amorphous state.

These results indicated that GRS had both crystalline and amorphous areas in the granules. The diffractograms of nGRS had shown definite diffraction peaks and possibly reflected a crystallinity region in the starch granule with 22.44% crystallinity. BM-GRS was obtained from varying milling conditions. During low milling speed (100 rpm) [Figure 6a], granules had similar crystallinity patterns with crystallinity 20.60, 20.53, and 20.48% at 30, 75, and 120 min, respectively. At an increased speed representing medium conditions (250 rpm) [Figure 6b], and at 30 min, the crystallinity peaks remained similar to the nGRS with 19.85% crystallinity. Increasing the milling duration to 75 and 120 min, with the medium speed conditions (250 rpm), the crystallinity peaks at 17°, 18°, and 23° disappeared. Moreover, percent crystallinity decreased to be 16.56 and 14.67%, respectively. When milling time was increased, the regions of amorphicity become larger. Finally, after increasing the speed to maximum velocity (400 rpm) [Figure 6c], the crystallinity peaks at 17°, 18°, and 23° disappeared at 30 min, and the amorphous region was predominant. Percent crystallinity was calculated as 14.46, 11.91, and 11.77% at 30, 75, and 120 min, respectively. Changing the conditions to a fixed milling time and varied speed [Figure 6d-f], affected the results. After a short milling time (30 min), the crystallinity of the GRS remained as 20.06 and 19.85% at speed 100 and 250 rpm, respectively. When the milling duration was increased to 75 and 120 min, the crystallinity of the GRS disappeared as the speed level increased past 250 rpm.

A milling speed of 100 rpm did not provide enough force to break the starch granule. Hence, the crystallinity pattern persisted, even at the longest milling time. When increasing the milling speed to medium level (250 rpm), the forces were enough to break the starch granules. The GRS started changing, and their polymorphism was obviously shown in the XR diffractogram when the milling time reached 75 min. Therefore, the alteration of crystallinity was predominantly dependent on the milling speed and milling time was a supplementary factor that synergized the polymorphic change. This result is concordant with the previous explanations in SwC (Y_1), %CWS (Y_2), %Gel (Y_3), and particle size (Y_4), which showed the major impact of the milling speed and minor impact of the milling time. The increasing of milling speed leads to an increase in amorphicity. The increase of percent gelatinization is related to the loss of crystallinity.[30,31] Therefore, increasing the milling speed induced the reduction of crystallinity and promoted the percent gelatinization. Moreover, the loss of crystalline order induced the penetration of free water to the starch molecule, thus increasing %CWS.[23]

CONCLUSION

The effect of milling speed and time on the physical characteristics of nGRS was examined using 2-factor-3-level factorial design. Relationships were found between those two parameters and six physicochemical responses. Four responses were obtained as mathematical equations; SwC, CWS, percent gelatinization, and particle size. The milling speed significantly affected all responses, while the milling time significantly affected %CWS and particle size in a positive manner. This meant that increasing milling speed significantly increased all four properties ($P < 0.05$). Increasing the milling time significantly increased only %CWS and particle size ($P < 0.05$). There was only one positive interaction effect of milling speed and time on %CWS. This indicated that the effect of speed on

the CWS varied according to the change in milling duration. The quadratic term of milling speed (X_1^2) significantly affected SwC in a negative manner and particle size in a positive manner. At the initial milling speed, the SwC began to increase until the milling speed reached a certain velocity when the SwC would begin to decrease. Particle size was conversely influenced. At the initial milling speed, the particle size began to decrease until the milling speed reached a certain velocity, whereupon the particle size would increase. The information from X-ray diffractogram and SEM analysis were supportive and in complete concordance with the previous results. They were used to explain and confirm all the results from DoE models.

All responses were significantly dependent on the milling speed. The milling time was a supplementary factor affecting the physical properties of the GRS. These factors could be varied in order to get BM-GRS with intended physical characteristics. The findings will be beneficial for the potential future modification of GRS using a ball mill.

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