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Effect of ultrasonic treatments on nanoparticle preparation of acid-hydrolyzed waxy maize starch

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ABSTRACT

Waxy maize starch was dispersed (14.7% solids) in an aqueous sulfuric acid solution (3.16 M), and hydrolyzed by stirring for up to 7 days at 40 °C with ultrasonic treatments at different vibration amplitudes (20 and 40%) and durations (30 and 60 min/day). The amount of starch nanoparticles in the hydrolyzates isolated after 7 days, measured by a dynamic light scattering detector, was raised from 20% to 70% by an ultrasonic treatment (20% amplitude, 30 min). The aggregation of nanoparticles possibly occurring during the hydrolysis was effectively prevented by the ultrasonication. Alternatively, ultrasonic treatments were applied to the re-dispersed suspension of the large microparticles of starch hydrolyzates (2 days) precipitated by a mild centrifugation (500 rpm, 10 min). By an ultrasonic treatment at 60% vibration amplitude for 3 min, the microparticles could be completely transformed to nanoparticles. The inherent crystalline structure of waxy maize starch (A-type in X-ray diffraction) remained after the ultrasonic treatments during acid hydrolysis, but it was disrupted by the ultrasonic treatments for the re-dispersed microparticles.

1. Introduction

Starch is an inexpensive and renewable biopolymer that is synthesized in granule shapes mainly from the photosynthesis in plants. Starch granules are composed of nano-sized blocklets that have semi-crystalline arrangements of starch chains (Oates, 1997). Crystalline lamellae exist in the blocklets with packed organization of amylopectin double helices that are formed by intertwining chains (more than 10 glucose units), whereas the branching points of amylopectin are mainly located in the amorphous lamellae (Gallant, Bouchet, & Baldwin, 1997). It has been reported that, in general, the blocklets in B- and C-type crystalline starches are larger (400–500 nm) than those in A-type crystalline starches (25–100 nm) (Gallant et al., 1997).

Starch nanoparticles, which might be sub-entities of the starch blocklets, can be readily obtained by hydrolyzing native starch granules by using acids or enzymes. The crystalline lamellae in starch granules are more resistant to acidic or enzymatic hydrolysis than the amorphous regions (Robin, Mercier, Charbonnière, & Guilbot, 1974). It was recently reported that the nanoparticles having crystalline structure (nanocrystals) obtained by acid hydrolysis with waxy maize starch showed a platelet shape with diameters ranging from 15 to 40 nm (Putaux, Molina-Boisseau, Momaur, & Dufresne, 2003). In the case of potato starch, however, nanocrystals of different sizes (40–100 nm) and shapes (round and grape-like) were obtained (Chen et al., 2008; Namazi & Dadkhah, 2008). The starch nanoparticles could be prepared in different morphologies and crystal structures according to the starch origin and manufacturing process.

In recent years, nanocrystals based on biopolymers have drawn considerable attention as novel and biofunctional materials in diverse industries. For example, in drug delivery systems, polysaccharide nanocrystals may exhibit prominent sustained release profiles, with assured safety (Lin, Huang, Chang, Feng, & Yu, 2011). Nanomaterials prepared from chitin and chitosan have proved effective for nanoencapsulation or for stabilizating nanoemulsions against coalescence (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011). Starch nanoparticles, particularly those formed from waxy maize, have been recommended as a candidate of reinforcing nanofillers in plastics (Le Corre, Bras, & Dufresne, 2010). When added to a polymeric matrix, such nanoparticles improved not only the mechanical strength but also the biodegradability of host polymer matrix (Kristo & Biliaderis, 2007). Although, utilization of starches originating from other sources such as potato (Chen et al., 2008) and pea (Yu et al., 2008) has been reported for nanocrystal formation, most reported studies have been performed using

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Table 1
Specific energy of ultrasonication and temperature of starch suspensions before and after ultrasonication at different vibration amplitudes and times.

Vibration amplitude (%)	Ultrasonic time (min)	Specific energy (J/cm^3)	Initial temperature (°C)	Temperature after treatment (°C)
20	30	67	40	41
	60	134	40	42
40	3	221	18	46
	30	137	40	47
	60	269	40	48
60	2	256	18	49
	2.5	312	18	50
	3	367	18	52
90	3	570	18	58

waxy maize starch. Furthermore, all of these attributes rely on the dispersion characteristics of the nanoparticles in the host polymer matrix. So far, the practical application of starch nanoparticles in nanocomposites has been restricted by their strong tendency to aggregate (Dufresne, 2008). In a previous study (Kim, Lee, Kim, Lim, & Lim, 2012), it was found that obtaining individual nanoparticles from starch was almost impossible regardless of the origin of the starch because of the strong tendency to produce aggregates of microparticles.Ultrasound energy can be transferred to starch through a process called cavitation, which refers to the formation, growth, and violent collapse of cavities in water. The energy provided by cavitation is approximately 10–100 kJ/mol, which is within the energy level of hydrogen bonds (Tischer, Sierakowski, Westfahl, & Tischer, 2010). Ultrasonication thus is particularly effective in breaking up the aggregates of nanoparticles formed through hydrogen bonds, thereby reducing the size and polydispersity of nanoparticles (Grieser, Ashokkumar, & Sostaric, 1999). Although acid hydrolysis has been commonly used in preparing nanoparticles from starches, no study has been reported on the hydrolysis process combined with a physical treatment such as ultrasonication for the formation of uniform dispersion of starch nanoparticles. In the present study, the effect of ultrasonic treatments on nanoparticle preparation from waxy maize starch induced by acid hydrolysis was examined. The physical properties of starch nanoparticles, including their size distribution and crystalline structure, were also investigated.

2. Materials and methods

2.1. Materials

Waxy maize starch was provided as a gift from Samyang Genex Inc. (Seoul, Korea). Sulfuric acid was purchased from Junsei Chemicals (Tokyo, Japan).

2.2. Preparation of starch nanoparticles

Starch nanoparticles were prepared using a modification of the procedure described by Angellier, Choisnard, Molina-Boisseau, Ozil, and Dufresne (2004). In brief, native waxy maize starch (44.07 g, dry solids) was dispersed in a diluted sulfuric acid solution (3.16 M, 300 mL), and the dispersion was stirred by a magnetic stirrer (200 rpm) at 40 °C. After various durations of hydrolysis, a sample aliquot was taken and neutralized with 1 M sodium hydroxide and centrifuged (3500 rpm, 10 min). Deionized water (300 mL) was added to the precipitates, and the mixture was stirred for 30 min at room temperature. The starch precipitates were recovered after a centrifugation (3500 rpm, 10 min). This washing process was repeated twice to remove the residual salts.

Subsequently, the starch precipitates isolated from the acid hydrolysis and washing process were re-dispersed in deionized water (300 mL), and the suspension was then mildly centrifuged at 500 rpm for 10 min to separate the solids in the supernatant and those in the precipitates. It was assumed that starch nanoparticles were isolated mainly in the supernatant whereas insufficiently hydrolyzed starch residues and nanoparticle aggregates were separated in the precipitates.

2.3. Ultrasonic treatment of starch suspensions

Two different ultrasonic treatments were applied to the starch suspension. In the first treatment, ultrasonication (once a day) was applied to the starch suspension (14.7% solids) during the hydrolysis at 40 °C. Different vibration amplitudes (20 and 40%) and durations (30 and 60 min) for the ultrasonication were applied for comparison by using a cylindrical titanium alloy probe of ultrasonic processor (VCX 750, Sonics and Materials Inc., Newtown, CT, USA, 750 W, 20 kHz). During the ultrasonic treatment, on/off pulses of 30/10 s were used to minimize temperature increase. In the second treatment, ultrasonication was applied to the re-dispersion (14.7% solids) of the starch precipitates isolated from a starch hydrolyzate (2 days' hydrolysis) by mild centrifugation (500 rpm and 10 min). Ultrasonication was conducted at different vibration amplitudes (40–90%) over different durations (2.0–3.0 min) at 25 °C. The actual powers delivered to the starch suspension were calculated from an equation: specific energy (J/cm^3) = energy output (J)/liquid volume (cm^3) (Nguyen, Rouxel, Hadji, Vincent, & Fort, 2011). The energy outputs which were varied by the percent amplitudes were recorded in the ultrasonic processor. The temperatures of the suspension before and after the ultrasonic treatments were measured (Table 1). The temperature was increased by the treatments, which was most significant at the highest amplitude (90%).

2.4. Yields of starch nanoparticles

The yields of the starch nanoparticles after acid hydrolysis were calculated as the percent ratio of insoluble particles after centrifuging the hydrolysis dispersion based on the initial dry starch weight.

2.5. Particle size distribution

The hydrodynamic particle size distribution was measured by dynamic light scattering (Dynapro Titan, Wyatt Technology, Santa Barbara, CA, USA). The hydrolyzed suspensions or re-dispersions were diluted with water to 0.1% and then transferred into a plastic disposable cuvette for measurement (Eppendorf, Germany). The refractive index and the viscosity of water were 1.333 and 1.00 cP at 20 °C, respectively.

2.6. Light transmittance (%)

The hydrolyzed starch suspensions or re-dispersions of the hydrolyzate precipitates (14.69%, w/v) were introduced into a glass cuvette (Hellma, Germany), and the light transmittance of the suspension was determined by measuring the absorbance at 640 nm

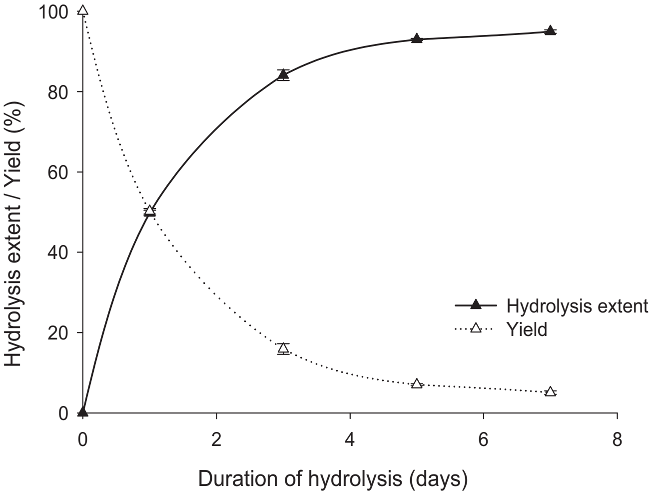

Fig. 1. Degree of hydrolysis and yield of starch hydrolyzates for a waxy maize starch dispersion (14.7% starch solids) in a diluted sulfuric acid (3.16 M) at 40 °C.

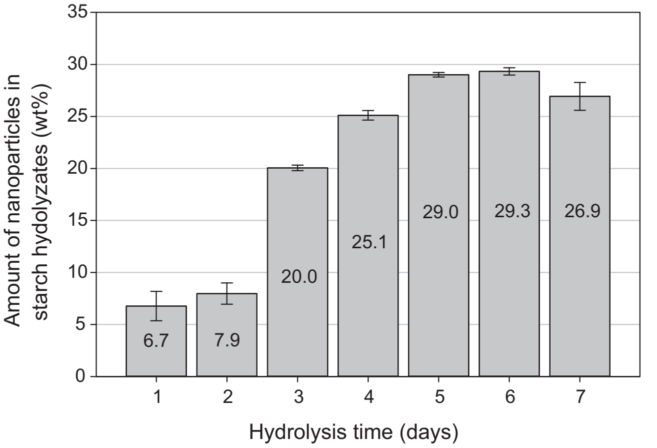

Fig. 2. Amount of starch nanoparticles in total starch hydrolyzates isolated by centrifugation (500 rpm, 10 min).

using a Shimadzu UV–vis spectrophotometer (UV-1700, Kyoto, Japan). Distilled water was used as a reference.

2.7. X-ray diffraction

The crystalline structure of starch nanoparticles dehydrated by freeze-drying was analyzed by using an X-ray diffractometer (Philips PW 3020, the Netherlands) with Cu Kα radiation ($\lambda = 1.54056$ Å) at a target voltage and current of 40 kV and 30 mA, respectively. The scanning range and rate were 5–30° (2θ) and 1.0°/min, respectively. Native waxy maize starch was also tested for comparison.

2.8. Morphology

The morphology of the starch nanoparticles was observed by a field emission scanning electron microscopy (FE-SEM, S-4700, Hitachi, Japan) at an accelerating voltage of 10 kV. Powdered starch nanoparticles were spread onto the copper grids coated with carbon supported film and a thin Au—Pd conductive coating was then used to reduce the charge effect.

3. Results and discussion

3.1. Isolation of starch nanoparticles

The degree of hydrolysis of waxy maize starch in an acid solution (3.16 M H_2SO_4) and the corresponding yields during the hydrolysis are shown in Fig. 1. The starch was rapidly hydrolyzed in the early stage up to 5 days but the hydrolysis became slow thereafter. The yields of starch hydrolyzates became less than 10% at 5 days, and reached to 5.05% at 7 days. It was hypothesized that the acid hydrolysis initially occurred mainly in the amorphous regions, followed by erosion of the crystalline regions in starch granule. This phenomenon has already been reported in several studies (Biliaderis, Grant, & Vose, 1981; Jayakody & Hoover, 2002; Kim et al., 2012; Robin et al., 1974). The hydrolysis process of starch is a "releasing process" of nanomaterials from microparticles. Thus, the resulting hydrolyzates consist of both micro and nanoparticles depending on the extent of hydrolysis (Le Corre, Bras, & Dufresne, 2011). However, the presence of heterogeneous particles may make the practical use of the particles difficult. Uniform nanoparticles in the dispersion are ideal to ensure good solution behavior and low surface roughness of the nanocomposites (Eschbach et al., 2007).

Centrifugation is one of the common techniques used to isolate starch nanoparticles from dispersions of starch hydrolyzates. In the present study, hydrolyzed starch solids were isolated as precipitates by centrifuging the dispersion of starch hydrolyzates at 3500 rpm for 10 min. The total amount (%) of starch solids isolated from the hydrolyzates decreased from 50.2% to 5.05% as the hydrolysis time increased to 7 days (Fig. 1). The isolated starch solids isolated after 2 days' hydrolysis were then re-dispersed in water and the dispersion was centrifuged again at a low speed (i.e. 500 rpm for 10 min) to separate nanoparticles and microparticles. It was confirmed that starch nanoparticles resided mainly in the supernatant, and microparticles existed in the precipitates (data not shown). It was also found that the amount of starch in the supernatant (starch nanoparticles) after the mild centrifugation increased from 6.7% to 29.3% based on starch hydrolyzates (Fig. 2). Therefore, the amount of starch nanoparticles increased when the hydrolysis time increased reaching near 30% of the starch hydrolyzates isolated, indicating that fragmentation of starch granules occurred continuously during the hydrolysis process (Le Corre et al., 2011). Although starch nanoparticles could be selectively isolated in the supernatant, the majority of the starch hydrolyzates (approximately 70%) resided in the precipitates as micro-sized particles. Herein, a question arose if the starch particles in the precipitates really were macro-sized and denser than the nanoparticles. Liu, Wu, Chen, and Chang (2009) stated that starch nanocrystals tend to form micro-scale aggregates through Van der Waals forces and hydrogen bonds, which could readily sediment as precipitates. If possible, therefore, when the aggregates are dissociated by a proper physical treatment such as ultrasonication, the yield of starch nanoparticles could be increased (Grieser et al., 1999).

3.2. Effect of ultrasonic treatment

During hydrolysis process, starch nanoparticles are continuously produced as starch granules are fragmented by acid, but resulting starch nanoparticles may tend to form aggregates that readily sediment as microparticles. To retard the aggregation and/or to dissociate the nanoparticles, ultrasonic treatments were applied to the starch dispersions, and changes in the size distribution of starch particles were examined by dynamic light scattering.

Fig. 3 depicts the ultrasonication effect on the particle size distribution of the hydrolyzed starch particles at different stages of hydrolysis (3, 5, and 7 days). The ultrasonic treatments varied from 20 to 40% (A20 to A40) in amplitude, and from 30 to 60 min (T30 to T60) in duration. Without ultrasonication, the

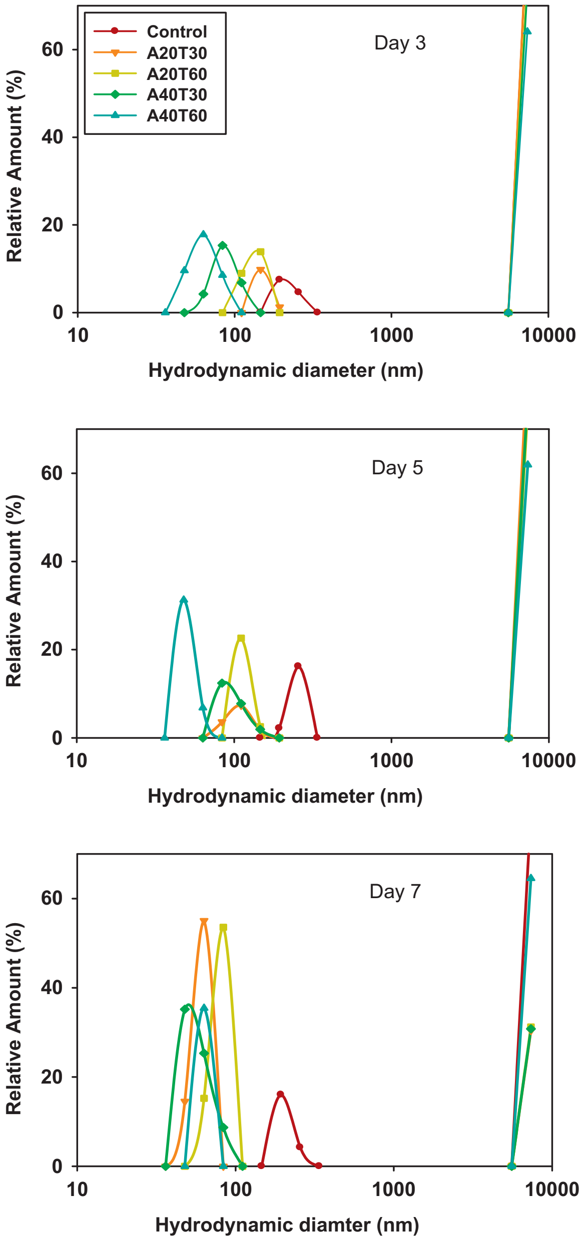

Fig. 3. Effect of ultrasonic treatments on particle size distribution of starch hydrolyzates at different hydrolysis times up to 7 days. Ultrasonication was conducted at different vibration amplitudes (20 and 40%) over different durations (30 and 60 min) once a day.

starch hydrolyzates showed bi-modal distributions consisting of microparticles (>2 μm) and nanoparticles (<200 nm). In agreement with the data shown in Fig. 2, the amount of nanoparticles increased as the hydrolysis continued. The bi-modal distribution of starch hydrolyzates was also reported in the recent electron microscopy study of Le Corre et al. (2011).

The portion of small starch particles (<200 nm) was not much different by the mildest ultrasonication (A20T30) when the hydrolysis period was 3 days (Fig. 3), although the average size of the small particles was reduced from 280 to 140 nm (data not shown). With higher doses for ultrasonication, however, the portion of nanoparticles increased with continual decreases in average particle size, indicating that the aggregates of starch nanoparticles were effectively dissociated by the ultrasonic energy. For the starch hydrolyzates obtained after 3 days, the highest dose of ultrasonication (A40T60) reduced the average size of the total starch hydrolyzates to less than 100 nm and substantially increased the portion of nanoparticles. A higher vibration amplitude increases the intensity of the bubble collapse and consequently, the deaggregation of the nanoparticle clusters became more effective (Mason & Lorimer, 2002). Similar results have been reported for cellulose fibers by Chen et al. (2011), who found that the degree of nanofibrillation depended on the output power of ultrasonic treatment. The cellulose nanofibers became more uniform and slender with increased intensity of ultrasonication. Similar effects were obtained by increasing the ultrasonic time. When the treatment was prolonged from 30 to 60 min at 20% amplitude, the portion of starch nanoparticles increased nearly to double.

The effect of ultrasonic treatment was more distinct when hydrolysis level was higher. When hydrolysis was done for 7 days, a substantial increase in the portion of starch nanoparticles was observed when the starch hydrolyzates were treated with the lowest dose of ultrasonication (A20T30). Under these conditions, starch nanoparticles (<100 nm) could be obtained at a high yield (approximately 70%). Starch granules that had been more extensively eroded by acids may have become less organized and more susceptible to the physical treatment. More likely, more starch nanoparticles had been produced by the higher degree of hydrolysis, which were simply released from the aggregates by the treatment. However, excessive ultrasonication (A40T60) resulted in an opposite effect on the size distribution, resulting in decreasing the amount of nanoparticles (Fig. 3). It indicates that the excessive treatment may induce aggregation through the secondary interactions between the individual nanoparticles. A similar result was reported by Kim, Park, and Lim (2008) for waxy rice starches hydrolyzed to α-amylase. In addition, the trend of re-aggregation was also observed in starch nanoparticles by Sauter, Emin, Schuchmann, and Tavman (2008). They suggested that an increase in vibration amplitude induced the bubbles to grow so large that the time available in the adjacent rarefaction cycle became insufficient for them to collapse, which reduced the tendency of cluster breakage.

Increases in the vibration amplitude and time of ultrasonication shifted the size peaks of small starch particles toward the range of smaller sizes. However, the extents of size decrease were not always proportional to the levels of amplitudes and time applied. The changes in average particle size and yield of nanoparticles were highly depended on the extent of acid hydrolysis. For the starch that was mildly hydrolyzed (3 days), size reduction continued as the dose of ultrasonic treatment increased, whereas for the starch excessively hydrolyzed for 7 days, the average size became 70 nm with the lowest dose of ultrasonic treatment (A20T30) but not further changed by increasing the ultrasonication dose. The amount of starch nanoparticles showed similar trends, with continual increase when the hydrolysis time was 3 days, but maximum at the lowest dose of ultrasonication with the excessively hydrolyzed starch. Temperature of starch suspensions could rise due to the absorption of ultrasound energy (Table 1). To minimize the heating, on/off cycles of ultrasonication were used in this study. The temperature increase might cause the swelling of hydrolyzed starch particles (El'Piner, 1964; Suslick, 1988). The swelling of starch particles may assist the formation of aggregates, which resulted in the size increase as observed for starch hydrolyzates treated by excessive ultrasonication. In this study, however, the swelling of starch particles might be somewhat suppressed by the presence of sodium sulfate (Na_2SO_4) produced by the neutralization of the starch suspension (Oosten & Breda, 1990).

The overall results clearly proved that ultrasonic treatment during acid hydrolysis of starch was effective in producing starch nanoparticles. It may prevent the aggregate formation of starch nanoparticles and/or dissociate the nanoparticles from the aggregates. To obtain the maximal yield of nano-sized starch particles,

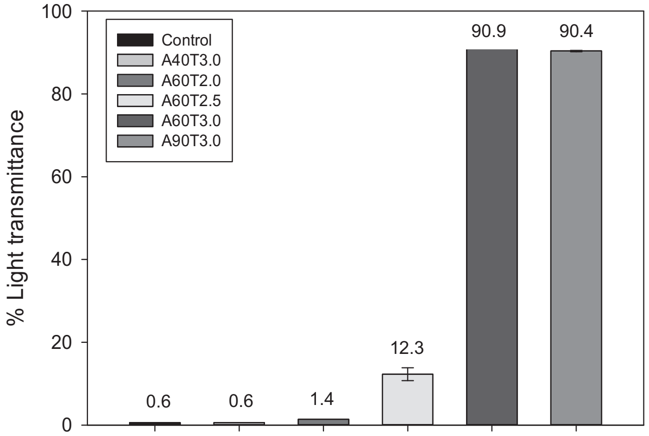

Fig. 4. Effect of ultrasonic treatments on light transmittance of re-dispersed suspensions of precipitates of starch hydrolyzates (14.7% solids, 2 days' hydrolysis). Ultrasonication was performed at 40–90% amplitude (A) and for 2.0–3.0 min (T).

ultrasonication at 20% amplitude for 30 min, every day during the hydrolysis of starch for 7 days, could be suggested. For the starch hydrolyzates treated for 3 and 5 days, the ultrasonic treatments tested could raise the yield of starch nanoparticles to only 40%. However, because only approximately 5% of starch hydrolyzates were isolated after 7 days of hydrolysis, the maximal yield of starch nanoparticles (approximately 70%) resulted in only 3.5% of the overall yield from the initial amount of starch. Thus, further improvement in the process is necessary to increase the yield of starch nanoparticles.

3.3. Process improvement for starch nanoparticle preparation

The relative crystallinity of waxy maize starch has been reported to range from 30% to 50% (Cooke & Gidley, 1992; Jayakody & Hoover, 2002). If the hydrolysis process used in this study was supposed to only erode the amorphous regions of the starch (50–70% of the starch), the hydrolysis for 2 days resulting in a recovery yield of 26.52% may be sufficient to remove the amorphous regions in the starch (Fig. 1). However, the hydrolysis process continuously decreased the recovery yield after 2 days, which suggested that hydrolysis occurred not on the amorphous regions but the crystalline regions of starch. The amount of starch nanoparticles in the recovered starch hydrolyzates was only approximately 8% after 2 days' hydrolysis, and became above 20% after 3 days (Fig. 2). Putaux et al. (2003) reported that the resistant residue of nano-sized platelets could be obtained after a long period of hydrolysis.

As shown in the previous results, more nanoparticles could be obtained through ultrasonic treatments, based on the assumption that some of the nanoparticles resided in the aggregates. Most of the starch nanoparticles were isolated in the supernatant whereas the aggregates may reside in the precipitates after a mild centrifugation of the dispersion of starch hydrolyzates at 500 rpm. The precipitates from the starch hydrolyzed for 2 days were re-dispersed in water (14.69 g/100 mL), and then the dispersion was treated by ultrasonication at different vibration amplitudes of 40%, 60%, or 90% for 3 min. The initial dispersion of precipitates prior to the ultrasonic treatment was opaque, but the light transmittance of the dispersion (clarity) became increased as the ultrasonic dose increased (Fig. 4). The dispersion became clear (>90%T) when the ultrasonic treatment was done at 60% amplitude for 3 min. With a lower amplitude (40%, A40T3.0) the light transmittance changed little indicating that the applied ultrasonic energy was not sufficient for breaking the aggregates. The increased transparency reflects that the starch aggregates or microparticles were broken down into smaller particles by the ultrasonic treatment. A similar result was reported by Liu et al. (2009), who observed that starch slurries were transformed from a mixture containing sediment to a gel or suspension as a result of a reduction of the particle size from 3–6 μm to 10–20 nm under a pressure of 207 MPa. Jenny, Kai, Raymond, Sandra, and Muthupandian (2009) found that after being treated by ultrasonication, a dispersion of waxy rice starch showed a decrease in viscosity and an increase in solubility and clarity. They suggested that ultrasound treatment facilitated the disintegration of starch granules and the formation of a homogeneous starch solution.

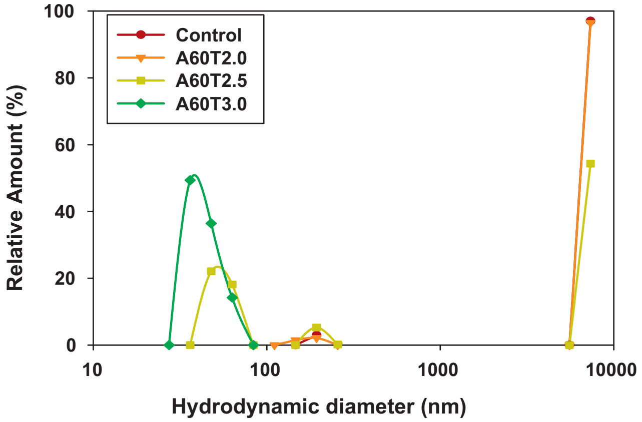

Fig. 5. Effect of ultrasonic treatments on particle size distribution of re-dispersed suspension of precipitates of starch hydrolyzates (14.7% solids, 2 days' hydrolysis).

At 60% vibration amplitude, the light transmittance of the starch dispersion increased proportionally as the ultrasonication time increased (Fig. 4). This trend matched closely to the change in particle size distribution (Fig. 5). Ultrasonication for 2 min showed a similar size distribution to the control with a major microparticle peak (>2 μm) and small nanoparticle peak (200–300 nm), which suggests that the treatment was insufficient to dissociate the aggregated particles. When the treatment was prolonged to 2.5 min, the peak intensity of the microparticles substantially decreased to approximately 50% of the initial value, and new peak appeared at 50–80 nm. With 3 min of ultrasonication at the same amplitude, the peak for starch nanoparticles moved to a smaller size range, and its intensity was further increased reaching nearly 100% of the total starch particles in the dispersion. These starch nanoparticles possibly represented starch blocklets which had resided in the granules (Gallant et al., 1997).

From the results, it was confirmed that individual starch nanoparticles were able to produce at a yield of nearly 100% by using a mild acid hydrolysis combined with ultrasonication process. As shown in Fig. 5, the starch hydrolyzates recovered after acid hydrolysis for 2 days (26.52% yield) could be completely transformed to nanoparticles by a post-ultrasonic treatment (60% amplitude, 3 min). It was assumed that the ultrasonic treatments behaved as a dissociation force of the nanoparticles clustered in the aggregates. Dual treatments of ultrasonication to both the starch dispersion during hydrolysis, and the dispersion of the microparticle precipitates may alternatively be used to improve the preparation process of starch nanoparticles.

3.4. Crystallinity of starch nanoparticles

To investigate the effect of ultrasonication on the crystalline structure of starch nanoparticles, X-ray diffraction patterns were observed. Native waxy maize starch granules had a typical A-type crystalline arrangement, showing diffraction peaks at around 15°, 17°, 18° and 23° (2θ) (Fig. 6), in consistent with the previous findings (Cheetham & Tao, 1998; Zobel, 1964). The acid hydrolysis

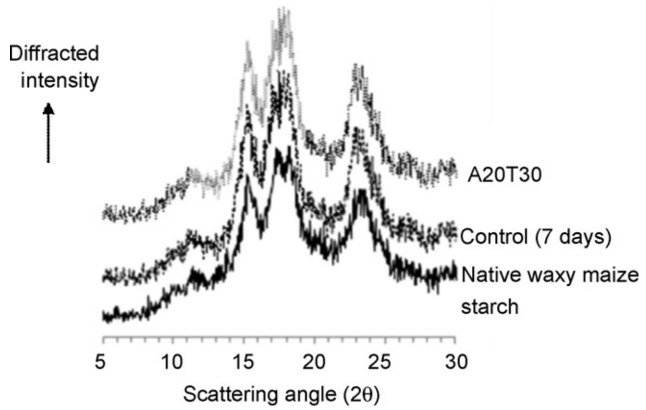

Fig. 6. X-ray diffractograms of starch hydrolyzates obtained by an acid hydrolysis (7 days, control) and an ultrasonic treatment during the hydrolysis at 20% amplitudes for 30 min.

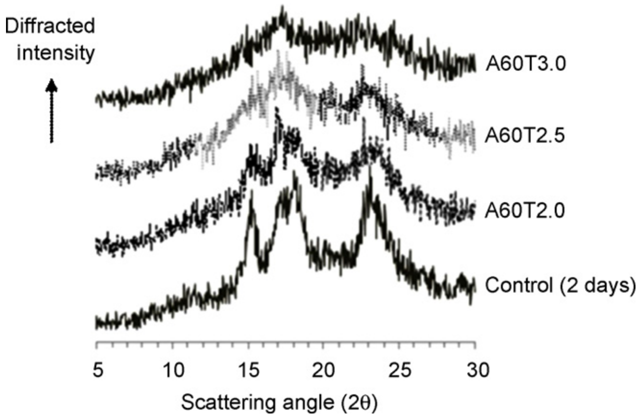

Fig. 7. X-ray diffractograms of starch hydrolyzates obtained by an acid hydrolysis (2 days, control) and ultrasonic treatments at 60% amplitude for 2.0–3.0 min with the re-dispersed suspension of precipitated hydrolyzates.

may increase the degree of crystallinity, because it was assumed to preferentially cleave the starch chains residing in the amorphous regions. Simultaneously, reordering of the chain segments to a more ordered arrangement (annealing) may occur as the mobility of the starch chains was enhanced by hydrolysis (Kainuma & French, 1971). Acid hydrolysis for 7 days (Control in Fig. 6) resulted in a slight increase in the peak intensity in the X-ray diffraction pattern. Mild ultrasonication (A20T30) during acid hydrolysis induced an additional increase in crystallinity based on the diffraction pattern. The increases in peak intensity by the acid hydrolysis and ultrasonication were small, indicating that the erosion of starch granules induced by both treatments was not much selective for the amorphous regions of starch. The crystalline structures of starch hydrolyzates obtained after 3 days was not markedly changed by ultrasonication. However, starch hydrolyzates obtained after the prolonged time was more susceptible to ultrasonication (from A40T30), yielding the decrease in crystallinity (data not shown). The starch suspensions treated by ultrasonication (A20T30) for 7 days consisted of individual nanoparticles (Fig. 8a). They existed in round shape ranged in diameter from 50 to 90 nm, which may correspond to the starch blocklets (Gallant et al., 1997). It confirms that the ultrasonic treatments behaved as a dissociation force of the particles clustered in the aggregates.

When the precipitates were re-dispersed and subsequently treated by ultrasonication, disruption of the crystalline structure of the starch was obvious (Fig. 7). When the vibration amplitude was equally 60%, and the time increased to 3 min, the decrease in crystallinity together with peak disappearance positively depended on the treatment time. The peaks at 15° and 18° (2θ) totally disappeared, and only a small peak at 17° and 23° (2θ) remained when the ultrasonic treatment was done for 3 min. Recently Li, Yue, and Liu (2012) sonicated microcrystalline cellulose (MCC) and showed, under X-ray crystallography, that the crystallinity of the nanocrystalline cellulose (NCC) decreased with increasing ultrasonication time. Liu et al. (2009) claimed that the decrease of crystallinity of starch particles could be attributed to a reduced degree in the crystalline arrangement or excessively small crystallites by the reduction of particle size. The X-ray diffraction data revealed that the ultrasonication disrupted the long-ranged arrangement of starch chains in nanoparticles when the re-dispersed starch microparticles or nanoparticle aggregates were treated. With the size reduction, the partial loss of crystallinity may additionally contribute to the increase of clarity of the dispersion (Fig. 4). The temperature increases induced by the ultrasonic treatments might assist the decreases in crystallinity (Table 1). However, scanning electron microscopy revealed that the starch suspensions treated by ultrasonication consisted of well-defined nanoparticles (Fig. 8b). It indicates that the starch nanoparticles remained relatively intact although their crystalline arrangement was somewhat disrupted by the ultrasonic treatments. The morphology of starch nanoparticles was similar among the starch samples treated at different times

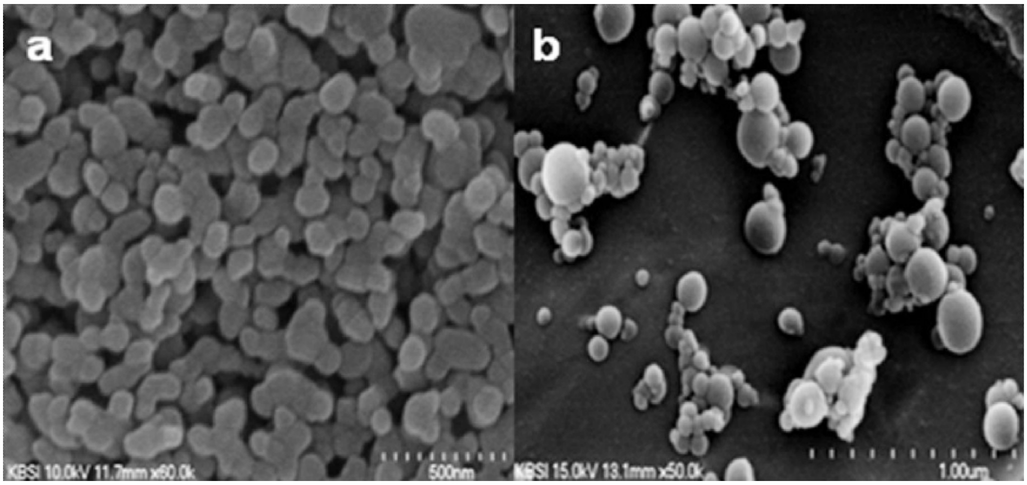

Fig. 8. FE-SEM images of the optimally prepared starch nanoparticles. (a) Acid hydrolysis for 7 days and an ultrasonic treatment during the hydrolysis at 20% amplitudes for 30 min; (b) acid hydrolysis for 2 days and an ultrasonic treatment at 60% amplitude for 3.0 min with the re-dispersed suspension of precipitated hydrolyzates.

(2–3 min) at 60% amplitude (data not shown). For a sample which had been optimally prepared (A60T3.0), nanoparticles existed in round shape with average diameter of 70 nm, although some large particles (around 120 nm) were mixed. This result was in agreement with a result of particle size distribution measured by light scattering (Fig. 5).

4. Conclusions

Mild acid hydrolysis combined with ultrasonication could effectively produce starch nanoparticles. Ultrasonication played on important role in dissociating the nanoparticle aggregates that may be formed during the hydrolysis, thereby effectively increasing the yield of starch nanoparticles. However, the starch nanoparticles treated by ultrasonication may reduce crystallinity of starch.

Acknowledgements

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