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Preparation of crystalline starch nanoparticles using cold acid hydrolysis and ultrasonication

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ABSTRACT

Waxy maize starch in an aqueous sulfuric acid solution (3.16 M, 14.7% solids) was hydrolyzed for 2–6 days, either isothermally at 40 °C or 4 °C, or at cycled temperatures of 4 and 40 °C (1 day each). The starch hydrolyzates were recovered as precipitates after centrifuging the dispersion (10,000 rpm, 10 min). The yield of starch hydrolyzates depended on the hydrolysis temperature and time, which varied from 6.8% to 78%. The starch hydrolyzed at 40 °C or 4/40 °C exhibited increased crystallinity determined by X-ray diffraction analysis, but melted in broader temperature range (from 60 °C to 110 °C). However, the starch hydrolyzed at 4 °C displayed the crystallinity and melting endotherm similar to those of native starch. The starch hydrolyzates recovered by centrifugation were re-dispersed in water (15% solids), and the dispersion was treated by an ultrasonic treatment (60% amplitude, 3 min). The ultrasonication effectively fragmented the starch hydrolyzates to nanoparticles. The hydrolyzates obtained after 6 days of hydrolysis were more resistant to the ultrasonication than those after 2 or 4 days, regardless of hydrolysis temperatures. The starch nanoparticles could be prepared with high yield (78%) and crystallinity by 4 °C hydrolysis for 6 days followed by ultrasonication. Scanning electron microscopy revealed that the starch nanoparticles had globular shapes with diameters ranging from 50 to 90 nm.

1. Introduction

Starch nanoparticles, which may represent starch blocklets in native granules, can be readily obtained by hydrolyzing native starch granules by using acids or enzymes. Putaux, Molina-Boisseau, Momaur, and Dufresne (2003) presented a possibility that nanoparticles of crystalline structure could be prepared by treating waxy maize starch suspension (5%) with 2.2 N HCl for an extended period (36 °C for 6 weeks), which had a platelet-shape with diameters ranging from 15 to 40 nm. Angellier, Choisnard, Molina-Boisseau, Ozil, and Dufresne (2004) improved the preparation process for starch nanoparticle by using H_2SO_4, and reported that the nanoparticles had different morphologies and sizes depending on the botanical origin of starch. High amylose maize starch with an amylose content of approximately 70% produced larger and more round-shaped nanoparticles than those obtained from waxy maize starch which has essentially no amylose (Kim, Lee, Kim, Lim, & Lim, 2012).

In recent years, crystalline nanoparticles (nanocrystals) originated from biopolymers have drawn considerable attention because they may be utilized as novel and biofunctional materials in diverse industries. For example, in drug delivery systems, polysaccharide nanocrystals have been suggested as carriers for safe and prominently sustained release (Lin, Huang, Chang, Feng, & Yu, 2011). Nanocrystals prepared from chitin and chitosan have been effective in nanoencapsulation and stabilization of nanoemulsions against coalescence (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011). Starch nanoparticles, particularly those prepared from waxy maize, have been recommended as reinforcing nanofillers in various plastics (LeCorre, Bras, & Dufresne, 2010). In a polymeric matrix, starch nanoparticles improved its mechanical strength as well as biodegradability (Kristo & Biliaderis, 2007).

However, starch nanoparticles obtained by the classic acid hydrolysis are limited for practical utilization because the nanoparticles have a strong tendency to aggregate especially when those are in the form of dry powders (Dufresne, 2008). Moreover, the hydrolysis usually requires an extended period (longer than 5 days) and the preparation yield from native starch is low, typically less than 20%. Therefore, several researches have been performed to produce starch nanoparticles applying physical treatments. A combined process of acid hydrolysis for a short period (2 days at 40 °C) followed by a facile ultrasonication (60% amplitude, 3 min) showed a similar effect (Kim, Han, Kweon, Park, & Lim, 2013). The preparation time became shortened and the ultrasonication effectively

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prevented the aggregation of starch nanoparticles. However, the ultrasonication readily disrupted the crystalline structure of starch, and the recovery yield of crystalline nanoparticles solely prepared by acid hydrolysis remained low (less than 30%). As a pure physical treatment process, a high-intensity ultrasonication (80% power output, 75 min) was applied to an aqueous dispersion of starch (Bel Haaj, Magnin, Pétrier, & Boufi, 2013). This process offered the advantage of being rapid and easy process for starch nanoparticle preparation. However, the physical treatment in this study also induced the complete disruption of starch crystallinity. It was assumed that the ultrasonic treatment exerted effects on the cluster structure inside starch granule, especially the crystalline region (Zhu, Li, Chen, & Li, 2012). If the starch nanoparticles can be preserved in crystalline structure similar to native starch after such physical treatment, the powder products could be readily obtained and their acceptability to industrial items such as composites as nano-fillers will be improved.

The crystallinity of native starch may be improved through two different processes. Selective erosion on the amorphous regions in starch granules using acids or enzymes is commonly practiced to raise the inherent crystallinity. A physical treatment such as heating with certain combinations of time, temperature and moisture, referred to annealing or heat-moisture treatment could increase crystallinity (Cameron & Donald, 1992). Various parameters in the process for starch nanoparticle preparation therefore may be modified to control the crystallinity of nanoparticles. The kinetic of acid hydrolysis is strongly dependent on the temperature and time, but not much on starch concentration (LeCorre, Bras, Choisnard, & Dufresne, 2012; LeCorre, Bras, & Dufresne, 2012). The hydrolysis rate of starch increases with temperature increase, but if the hydrolysis temperature is too high, starch crystals may decompose. Temperature cycling often improves the structural rearrangement to improve crystalline structure formation. It could induce stepwise nucleation and propagation promoting the growth of crystals and resulting in crystallinity increase (Wunderlich, 1976). Such a process is usually used for re-crystallization of starch chains in amorphous or low crystalline system, and thus may work differently during the hydrolysis for starch nanoparticle formation in which starch remains in solid state. However, starch chains become smaller by the hydrolysis and mobility of starch and tendency to associate may be increased. Therefore, it may be expected that structural rearrangement could happen more readily when the hydrolysis continues. Moreover, applying a low temperature like 4 °C might facilitate the association of starch chains, enhancing the improving the residual crystallinity or forming new crystalline structure in starch nanoparticles.

More recently, environmentally friendly approaches, i.e. high pressure homogenization (Liu, Wu, Chen, & Chang, 2009), extrusion (Giezen, Jongboom, Gotlieb, & Boersma, 2000), and ultrasonication (Bel Haaj et al., 2013) were investigated. However, all these physical treatments have a main drawback of partial or complete destruction of starch crystallinity. In the present study, starch hydrolyzates were obtained without disrupting crystallinity by using a hydrolysis under refrigerated conditions. The crystalline hydrolyzates recovered as precipitates after centrifugation were re-dispersed in water, and then subjected to an ultrasonic treatment to prepare nanoparticles. Recovery yield and morphological properties of the starch nanoparticles were investigated.

2. Materials and methods

2.1. Materials

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

2.2. Acid hydrolysis

Native waxy maize starch (44.1 g, dry solids) was dispersed in a diluted sulfuric acid solution (3.16 M, 300 mL), and the dispersion was magnetically stirred (200 rpm) under three different conditions: an isothermal condition at 4 or 40 °C, or a temperature cycle at 4 °C and 40 °C (4/40 °C, 1 day at each temperature) for 6 days. Hydrolysis reaction was performed in cold chamber (4 °C) or water-bath (40 °C), respectively.

At different periods of hydrolysis, sample aliquots (50 mL) were taken, neutralized by adding a sodium hydroxide solution (1 M), and centrifuged (10,000 rpm, 10 min). The wet precipitates were re-dispersed in distilled water (300 mL) by magnetic stirring for 30 min, and then the dispersion was centrifuged (10,000 rpm, 10 min). This washing process was repeated twice to remove the residual sodium sulfate salt.

2.3. Ultrasonic treatment of starch hydrolyzates

The aqueous dispersion of starch hydrolyzates was subjected to a subsequent ultrasonic treatment. Ultrasonication (VCX 750, Sonics and Materials Inc., Newtown, CT, USA, 750 W, 20 kHz) was performed at amplitude 60% for 3 min, which was equivalent to an energy input of 367 J/cm^3. The actual power delivered to the starch suspension was calculated from the following equation: specific energy (J/cm^3) = energy output (J)/liquid volume (cm^3) (Nguyen, Rouxel, Hadji, Vincent, & Fort, 2011). The temperature of the starch dispersion could rise due to the absorption of ultrasound energy. Therefore, on/off pulses of 30/10 s were applied during the ultrasonication to minimize heat generation. The temperature of the suspension after the treatment was approximately 50 °C.

2.4. Yield of starch hydrolyzates

The recovery yield of starch hydrolyzates after acid hydrolysis was calculated as the percent ratio of precipitated solids after centrifugation (10,000 rpm, 10 min) based on the initial weight of starch dry solids.

2.5. X-ray diffraction analysis

The crystalline structure of starch hydrolyzates and nanoparticles was analyzed with freeze-dried samples by using an X-ray diffractometer (X'pert APD, Philips, Netherlands) with Cu-Kα radiation (λ = 1.54056 Å) at a target voltage and current of 40 kV and 30 mA, respectively. The scanning range and rate were 5–40° (2θ) and 1.0°/min, respectively. The relative crystallinity (RC) of starch was quantitatively calculated following the method of Nara and Komiya (1983): $RC = A_c/(A_a + A_c)$, where A_c was the crystalline area, and A_a was the amorphous area on the X-ray diffractogram.

2.6. Thermal property

The thermal transition properties of starch hydrolyzates and nanoparticles were examined using a differential scanning calorimeter (DSC 6100, Seiko Instruments, Chiba, Japan). The instrument was calibrated with indium and an empty pan was used as the reference. The starch sample (3.0 mg, dry basis) and water (6.0 mg) were transferred to an aluminum DSC pan, which was then hermetically sealed and equilibrated at 4 °C for 2 h prior to analysis. The sample pan was scanned from 40 °C to 120 °C at a heating rate of 5 °C/min.

2.7. Particle size distribution

The hydrodynamic particle size distribution was measured by using a dynamic light scattering detector (Dynapro Titan, Wyatt Technology, Santa Barbara, CA). Samples were prepared by diluting the dispersions of starch hydrolyzates with distilled water to 0.1% (v/v) and then transferring them into a plastic disposable cuvette (Eppendorf, Germany) for measurement. The refractive index and the viscosity of water were 1.333 and 1.00 cP at 20 °C, respectively.

2.8. Scanning electron microscopy

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

3. Results and discussion

3.1. Acid hydrolysis

In a previous study (Kim et al., 2013), it had been confirmed that the starch hydrolyzates recovered after 2 days of hydrolysis could be mostly transformed to nano-sized particles by a post ultrasonic treatment (60% amplitude, 3 min). However, as the microparticles of starch hydrolyzates were fragmented to nanoparticles by the ultrasonication, the inherent crystallinity was almost completely removed. The present study, therefore, focused on the isolation of crystalline starch nanoparticles which were resistant to the subsequent ultrasonic treatment by using different temperature profiles: isothermally at 4 °C or 40 °C, and at cycled temperatures of 4/40 °C.

Fig. 1 shows the yields of starch hydrolyzates during the hydrolysis up to 6 days. The hydrolysis at 4 °C resulted in substantially higher yields of starch hydrolyzates compared to those from the high temperature hydrolysis at 40 °C. Limited heating for hydrolysis (25–50 °C) is typically practiced for the preparation of starch nanoparticles to prevent gelatinization but accelerate hydrolysis (Hoover, 2000). The recovery yields at 40 °C were between 6.8 and 23.3% whereas those at 4 °C were much higher (>78%) during the 6 days of hydrolysis. The temperature cycle at 4/40 °C displayed the intermediate yields between those of 4 and 40 °C. The low temperature hydrolysis was not effective in disintegrating the granule structure of starch

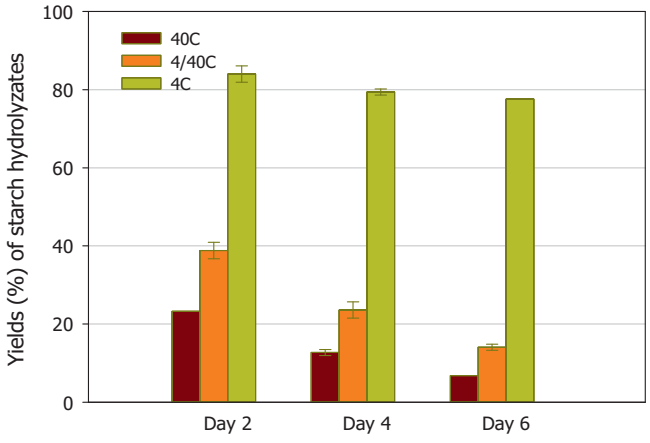

Fig. 1. Effect of hydrolysis temperatures on the recovery yield of starch hydrolyzates from waxy maize starch during an acid hydrolysis for 6 days, either isothermally at 4 °C and 40 °C, or at cycled temperatures at 4 °C/40 °C (1 day at each temperature).

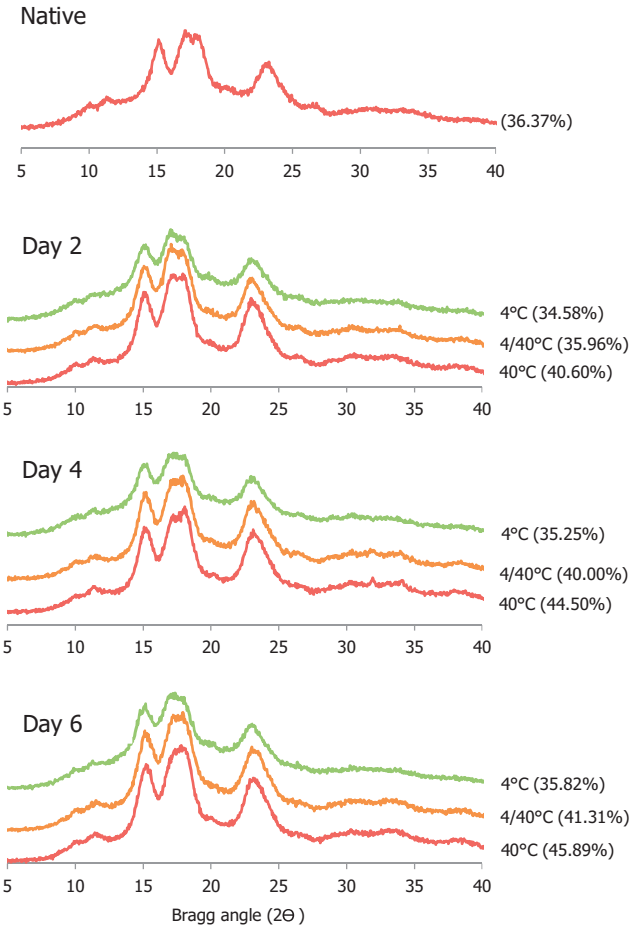

Fig. 2. Effect of hydrolysis temperatures on X-ray diffraction patterns of starch hydrolyzates, either at isothermally 4 °C and 40 °C, or at cycled temperatures at 4 °C/40 °C (1 day at each temperature).

. Strong dependency of starch hydrolysis on temperature has been reported (LeCorre, Bras, Choisnard, et al., 2012; LeCorre, Bras, & Dufresne, 2012). Higher yields when the temperature cycles were applied compared to those at 40 °C could be attributed to the cushioning effect of temperature cycling. By treating the starch suspension at 4 °C and 40 °C alternatively, the hydrolysis occurring at 40 °C would be suppressed when the temperature reached to 4 °C.

To investigate the effect of hydrolysis temperature on the crystalline structure of starch hydrolyzates, X-ray diffraction patterns of the dry powders of starch hydrolyzates were measured (Fig. 2). All the starch hydrolyzates exhibited diffraction peaks at around 15°, 17°, 18° and 23° (2θ) representing that those had typical A-type crystalline arrangements (Cheetham & Tao, 1998; Zobel, 1964). However, the level of the relative crystallinity of the starch hydrolyzates, based on the peak intensity, ranged from 34.6% to 45.9%. Differences in RC values between samples may reflect the fact that the hydrolysis conditions (temperature or time) influenced on the degree of the crystallinity. It was noteworthy that the level of crystallinity for the starch hydrolyzates prepared at the high temperature (40 °C) was higher than those at 4 °C, especially when the peak at Bragg angle of 23.0° (2θ) was compared. Moreover, the 4 °C hydrolyzate after 2 days displayed an XRD pattern showing the crystallinity slightly lower than that of native waxy maize starch. By extending the hydrolysis period up to 6 days, all starch hydrolyzates exhibited continuous increases in crystallinity, which was in agreement with the finding of Waigh, Kato, Gidley, Clarke, and Riekel (2000). However, the crystallinity increase induced by the acid hydrolysis was more significant during the early 4 days

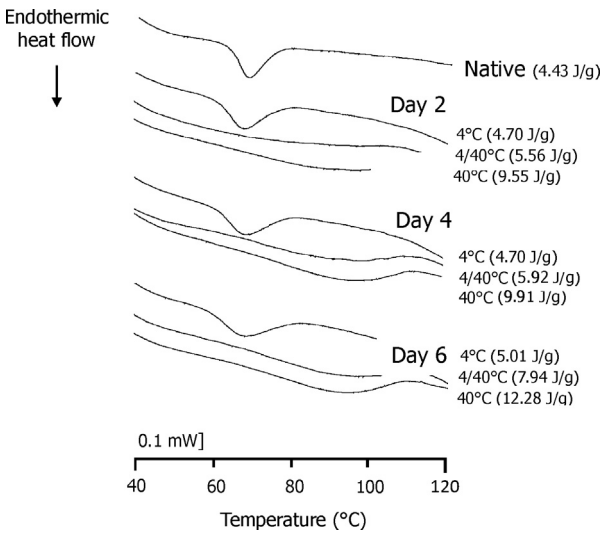

Fig. 3. Effect of hydrolysis temperatures on thermal transitions of starch hydrolyzates, either isothermally at 4 °C and 40 °C, or at cycled temperatures at 4 °C/40 °C (1 day at each temperature).

than those observed afterward. It was reported that the starch hydrolysis occurred more rapidly with higher selectivity to amorphous regions during the earlier stage of hydrolysis (Kim et al., 2012). Accordingly, the crystallinity increase was significant during the early period of hydrolysis in this experiment.

Crystal melting characteristics of the starch hydrolyzates obtained from different hydrolysis conditions were measured using a DSC (Fig. 3). The starch hydrolyzates treated at the high temperature (40 °C or 4/40 °C) displayed melting endotherms in broad temperature ranges from 60 °C to 110 °C. Similar results showing increased temperature and range for crystal melting has been reported by LeCorre, Bras, Choisnard, et al. (2012) and LeCorre, Bras, and Dufresne (2012), who assessed the influence of botanical origin, amylose content and crystalline type of starches on the thermal properties of starch nanocrystals. The increase of melting temperature and melting enthalpy by the mild acid hydrolysis might be attributed to the erosion of amorphous regions, resulting in a rigid crystalline structure. The melting enthalpy increased with an increase of the hydrolysis temperature and time (Fig. 3) in accordance with the relative crystallinity measured by X-ray diffraction analysis (Fig. 2). It was found that the 4/40 °C starch hydrolyzates showed lower melting enthalpy values (5.56–7.94 J/g) than those of the 40 °C hydrolyzates (9.55–12.28 J/g).

Unlike the starch hydrolyzates prepared at 40 °C and 4/40 °C, those obtained after the hydrolysis at 4 °C exhibited more pronounced and narrower endothermic peaks. The melting endotherm appeared similar to that of native starch, which might be attributed to the relatively low level of hydrolysis. The onset temperature for melting of the starch hydrolyzates was 65–68 °C which was similar to that of native starch, but the melting range for the hydrolyzates was slightly higher than that for native starch (21 °C vs. 12 °C). Moreover, the melting enthalpy was also higher for the starch hydrolyzates (5.02 J/g) than that for native waxy maize starch (4.43 J/g), indicating that more thermal energy was required for disrupting the amylopectin double helices in the starch hydrolyzates.

The crystallinity of native waxy maize starch was reported to range from 30% to 50% (Cooke & Gidley, 1992; Jayakody & Hoover, 2002). When the starch was hydrolyzed at 40 °C, the recovery yield became less than 30% even after 2 days of hydrolysis. If the hydrolysis occurred exclusively on the amorphous regions, the residual hydrolyzates after 2 days at 40 °C should have pure crystalline regions of the starch. However, the crystallinity increase observed by the XRD analysis was relatively minor (36.37% → 40.60%), and thus the hydrolysis during the 2 days of hydrolysis was not much selective on the amorphous regions. Kim et al. (2012) reported that the amorphous erosion occurred in the early stage of acid hydrolysis because of its physical accessibility, and the disruption of crystalline regions could follow in the late stage. Although the amorphous erosion was evident by the XRD data showing the increase of crystallinity within 2 days of hydrolysis (Fig. 2), the disruption of crystalline arrangement occurred concurrently during this period.

When the hydrolysis temperature was low (4 °C), the crystallinity was slight decreased after 2 days of hydrolysis (36.37% → 34.58%). Possibly the hydrolysis at the low temperature was not as selective as that at the high temperature. One possible explanation for this difference could be that the starch granules became swollen by the mild heating and thus the amorphous regions became exposed and susceptible to the action of acid. Therefore, the starch granules treated at 40 °C became hydrolyzed in more selective manner than those hydrolyzed at 4 °C.

Overall results from the XRD analysis show that the crystallinity of residual starch hydrolyzates correlates positively with the degree of hydrolysis. As the hydrolysis temperature and duration increased, the crystallinity of starch hydrolyzates became also higher. Based on the crystallinity changes with the variation of hydrolysis condition, it may be concluded that the hydrolysis was not actually selective to the amorphous regions, but it could occur at different levels to the amorphous and crystalline regions in starch granule, simply depending on their physical accessibilities.

In agreement with the XRD data, the starch hydrolyzates treated at 40 or 4/40 °C displayed broad melting endotherms with relatively high enthalpies (Fig. 3). The onset and end temperatures for melting were substantially higher than those of native starch and the low temperature hydrolyzates. If the crystalline structure of native starch remained unchanged after the hydrolysis, the melting peaks should have appeared in the temperature range similar to that of native starch. The shift to the higher temperature and wider range for melting indicates that those hydrolyzates had experienced some structural rearrangement, which was not detected by XRD analysis. The acid hydrolysis produced the starch chains of smaller molar sizes and thus greater chain mobility. During the hydrolysis, those starch chains might readily induce the structural rearrangement. One possible structural rearrangement affecting the crystallinity is annealing, which occurs typically at a temperature below that of gelatinization with excess water. When a native starch is annealed, melting endotherm of the annealed starch usually appeared at increased temperature within narrower range (Zavareze & Dias, 2011). However, in this experiment, starch was continually hydrolyzed and some of annealed structure could be disrupted by acid. Therefore, not only annealing but continuous acid action might result to the broadening of the melting endotherm. These structural rearrangements were expected to occur more readily when the hydrolysis temperature was higher, because the chain mobility was thermally enhanced. Accordingly, the starch hydrolyzates treated at the high temperature showed the broadened melting endotherms induced by these structural rearrangements.

The temperature-cycled hydrolysis had been attempted to accelerate those structural rearrangements because the association between starch chains was expected to be accelerated by treating the starch dispersion at the low temperature. The overall results, however, revealed that the temperature cycling was not much effective for improving the crystalline structure of the hydrolyzates. The hydrolysis processes used in this study preserved the crystalline structure of native starch at same or even higher degree of crystallinity, which might be desirable for the crystalline nanoparticle preparation using the subsequent ultrasonic treatment.

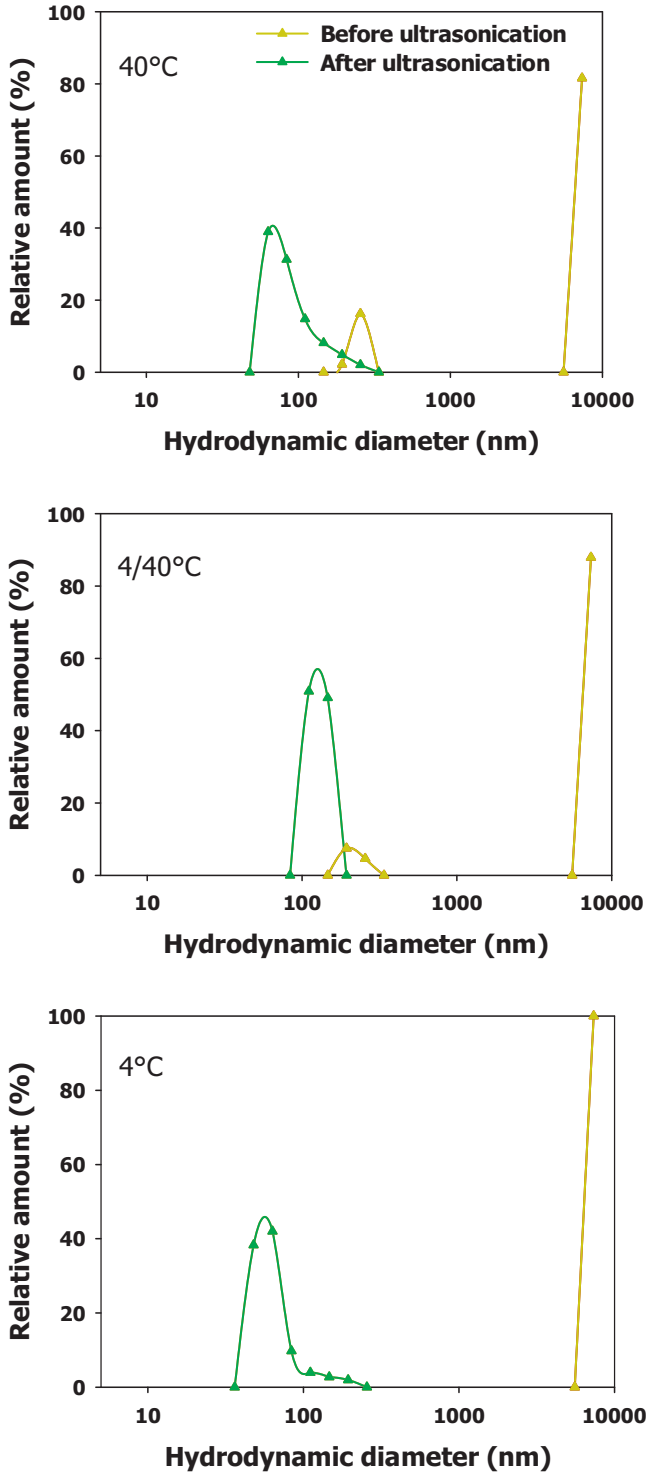

Fig. 4. Effect of ultrasonication (60% amplitude, 3 min) on particle size distribution of the starch hydrolyzates obtained under various hydrolysis temperatures for 6 days.

3.2. Ultrasonication

Fig. 4 shows the particle size distributions of starch hydrolyzates before and after the ultrasonic treatment. Starch hydrolyzates contained mostly microparticles prior to the treatment, but were completely transformed into nanoparticles by the ultrasonic treatment. The starch hydrolyzates prepared at 40 °C or 4/40 °C showed bi-modal distributions with both microparticles (around 5–7 μm) and nanoparticles (around 150–250 nm). Similar results have been reported in a previous study (LeCorre, Bras, & Dufresne, 2011), confirming that starch suspensions at any time of hydrolysis, both micro- and nano-sized particles could coexist. However, the starch hydrolyzates prepared at the low temperature (4 °C) contained only microparticles, because the degree of hydrolysis was apparently low (Fig. 1).

When the starch hydrolyzates were treated with the short and mild ultrasonic treatment (60% amplitude for 3 min, 367 J/cm^3), all the microparticles effectively fragmented to nanoparticles, regardless of the hydrolysis temperatures tested (Fig. 4). Other type of physical treatment such as high pressure homogenization has been used to produce starch nanoparticles (Liu et al., 2009). By the treatment, high amylose corn starch could be transformed to a gel or suspension as a result of reduction in particle size from 3–6 μm to 10–20 nm. More recently, Bel Haaj et al. (2013) found that starch nanoparticles could be prepared only by ultrasonic treatment of aqueous suspension of native starch for 75 min. This approach may be advantageous over the conventional hydrolysis process because starch nanoparticles can be produced by a simple and rapid physical treatment without the precedent hydrolysis using chemicals or enzymes. However, X-ray diffraction analysis for the starch nanoparticles revealed that the excessive ultrasonic treatment for the granule fragmentation removed the crystalline nature of starch too, providing amorphous nanoparticles.

Size distribution of the starch nanoparticles prepared by the ultrasonic treatment appeared slightly different among the starch hydrolyzates treated at different hydrolysis temperatures. Considering the fact that the polydispersity corresponded to the width of size distribution peak, the starch nanoparticles prepared by the hydrolysis under the temperature cycles (4/40 °C) followed by the ultrasonic treatment seemed to be more homogeneous than those after the hydrolysis under isothermal conditions (40 °C or 4 °C). However, the particle size of the starch sample treated by the temperature cycles appeared larger than those prepared under the isothermal conditions (Fig. 4).

The ultrasonic treatment increased the transparency (clarity) of starch suspension, as a result from the size reduction of suspended particles (data not shown). Jenny, Kai, Raymond, Sandra, and Muthupandian (2009) reported a similar result for a starch suspension treated by ultrasonication. They found that a dispersion of waxy rice starch showed a decrease in viscosity but increases in solubility and clarity. More specifically, electron microscopic observation verified that the mild hydrolysis and ultrasonic treatment produced well-defined nanoparticles (Fig. 5). Under the microscopy, the particle size was estimated to range from 50 nm to 90 nm for the starch nanoparticles treated at 4 °C or 4/40 °C. The particles had globular shapes which might correspond to the starch blocklets (Gallant, Bouchet, & Baldwin, 1997) which had inherently existed in native starch granules. The morphology of starch nanoparticles was exceptionally uniform and not affected by the hydrolysis conditions tested in this study. It strongly supports the claim that those nanoparticles represented the starch blocklets.

Ultrasonication influenced the crystalline structure of the starch hydrolyzates (Fig. 6). In a previous study (Kim et al., 2013), waxy maize starch hydrolyzed at 40 °C for 2 days exhibited a substantial disruption of crystalline structure by a subsequent ultrasonic treatment. In this study too, similar results with decreased crystallinity by ultrasonication were observed, especially for the hydrolyzates prepared at 4 °C (2.30%) or 4/40 °C (13.63%). The starch hydrolyzates treated for 4 days displayed XRD patterns with somewhat higher crystallinity than those treated for 2 days (2.72–15.88%), but still appeared highly disrupted by the ultrasonic energy. When the hydrolysis was done for 6 days, however, the crystallinity of starch hydrolyzates, regardless of hydrolysis conditions, appeared not much affected by the ultrasonic treatment (Fig. 6). This result

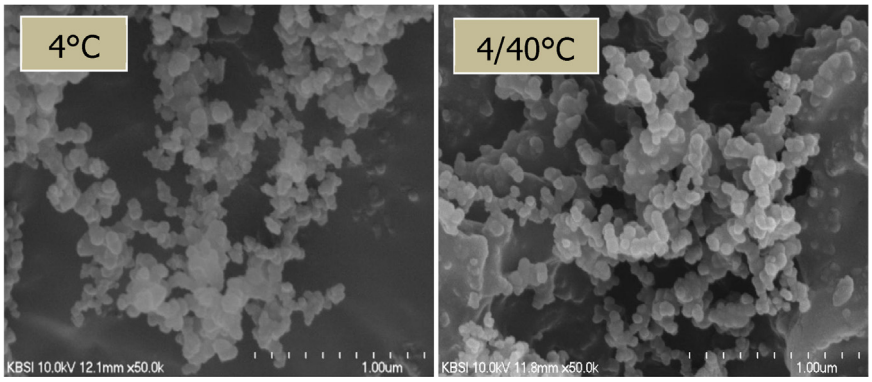

Fig. 5. FE-SEM images of starch nanoparticles obtained by acid hydrolysis at 4 °C or 4/40 °C for 6 days followed by ultrasonication.

revealed that the starch hydrolyzates became resistance to the ultrasonic treatment when the hydrolysis was sufficiently performed, for 6 days in this study. Particularly, the starch hydrolyzates obtained from the low temperature hydrolysis exhibited exceptionally high crystallinity (33.00%) after the ultrasonic treatment. In contrast to the low temperature hydrolyzates which were resistant to the ultrasonic treatment, those obtained at the high temperature either under the isothermal (27.68%) or cycled conditions (26.42%), displayed partial disruptions of their crystalline structure (Fig. 6 vs. Fig. 2). The degree of hydrolysis was higher as the higher temperature was applied for the hydrolysis, and thus the hydrolyzates prepared at the higher temperature became more susceptible to the physical treatment. It was found that the amount of soluble starch after the ultrasonic treatment was negligible (data not shown), indicating that the fragmented starch hydrolyzates could be mostly isolated after the ultrasonic treatment.

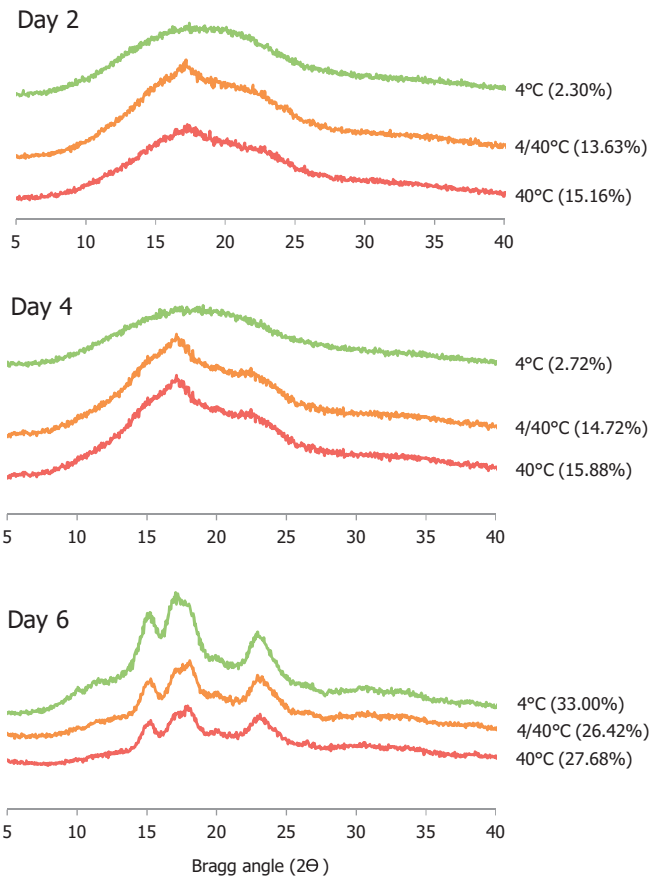

Fig. 6. X-ray diffraction patterns of starch nanoparticles obtained by acid hydrolysis for 6 days at various temperatures and ultrasonication.

The XRD results revealed that the ultrasonic treatment could be used as a tool to control the crystallinity of the starch. The changes induced by the ultrasonic treatment depended on the rigidity of starch sample. A large portion of starch (>80%) was dissolved after 6 days of hydrolysis when the temperature was high (40 °C or 4/40 °C), whereas only a small fraction (about 22%) was dissolved when the temperature was 4 °C (Fig. 1). Therefore it was expected that the residual starch after the low temperature hydrolysis contained more amorphous starch than those treated at the high temperature or cycled temperatures as shown in the XRD data (Fig. 2). Therefore, the high temperature hydrolyzates which had a high level of crystallinity were susceptible to the physical treatment due to the extensive hydrolysis, and thus the degradation of residual crystals by the physical treatment became obvious. However, the same physical treatment to the lower temperature hydrolyzates resulted in the low degree of structural disruption because the starch granules were still rigid and intact because the degree of hydrolysis was substantially low. These results may raise the question if the hydrolysis process is necessary. The study of Bel Haaj et al. (2013) demonstrates that raw starch tends to lose its inherent crystallinity by ultrasonication. Therefore, mild hydrolysis process is needed for protecting the crystalline regions against ultrasonication.

Starch nanoparticles formed by the prolonged hydrolysis (6 days) followed by the ultrasonic treatment exhibited more pronounced and narrower endothermic transitions, compared to those of the starch hydrolyzates prior to the treatment (Fig. 7). The samples prepared by 2 or 4 days of hydrolysis and ultrasonication exhibited no or smaller endotherms (data not shown), in accordance with the XRD data. After 6 days of hydrolysis followed by the ultrasonic treatment, the temperature range for melting was 11.4 °C for the 4 °C sample, 35.7 °C for the 4/40 °C sample, and 34.9 °C for 40 °C samples, respectively. The melting temperatures for the starch nanoparticles were also slightly increased by the ultrasonic treatment. The peak temperature was 73.3 °C for the 4 °C sample, 99.1 °C for the 4/40 °C sample, and 97.7 °C for the 40 °C sample. Based on

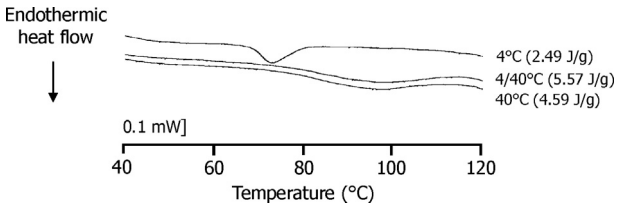

Fig. 7. DSC thermal transitions of starch nanoparticles obtained by an acid hydrolysis for 6 days at various temperatures and ultrasonication.

these DSC results, it was hypothesized that a portion of the amorphous regions was removed, or a minor rearrangement of starch chains occurred during the ultrasonic treatment. Considering the decrease in melting range, it was assumed that the ultrasonic treatment made the crystalline structure in nanoparticles more homogeneous. More likely, the size reduction as well as the increased uniformity by the ultrasonic treatment might contribute the crystalline melting in narrower range. After ultrasonication, it was also found that ΔH values for all samples were decreased, especially for 4 °C sample. As discussed previously, the ultrasonic treatment exerted effects on the crystalline regions in starch granule.

Overall results suggested a process for the preparation of starch nanoparticles with exceptionally high recovery yield (nearly 80%), which was an acid hydrolysis at 4 °C for 6 days followed by a mild ultrasonic treatment. Prior to the ultrasonic treatment, the starch hydrolyzates could be neutralized and washed off the residual salts by centrifugations. Therefore, starch nanoparticles could be obtained in dry powders simply by ultrasonic treatment and dehydration. By changing the hydrolysis temperature, starch nanoparticles could be obtained in different melting characteristics. In the case that thermal resistance is required for the utilization of starch nanoparticles, the acid hydrolysis at a high temperature such as 40 °C will be favorable although the recovery yield of nanoparticles is low.

4. Conclusions

A low temperature acid hydrolysis followed by a mild ultrasonic treatment could effectively produce crystalline nanoparticles from waxy maize starch. The crystallinity could be completely recovered from that of native starch to the nanoparticles, even though ultrasonication was performed. When the hydrolysis was insufficient, the crystalline structure of starch became susceptible to the ultrasonic treatment. The melting characteristics of starch nanoparticles could be varied according to the hydrolysis conditions. It can be concluded that a combination of low temperature acid hydrolysis and ultrasonic treatment is an effective process for the mass production of crystalline starch nanoparticles.

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