International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Formation of Starch Nanoparticles by a Combined Oxidation Process Involving Ultrasonics and Hydrogen Peroxide

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Abstract: ***A new technique for starch nanoparticle formation was developed by combining sonication with hydrogen peroxide addition. By a combination of H_2O_2 and sonication, it was expected to obtain starch particles in the range of 10-100 nm with the homogeneous distribution. In this study, the oxidation-sonication process applied with several process parameters, including power amplitude, time process, temperature, and starch concentration. The results showed that the higher amplitude, the bigger particle size, but the size still in range 100-1000 nm. The longer processing time promoted, the curve was shifted to a bigger particle size. At low temperature, H_2O_2 and the bubble of cavitation energy were moved easier and resulted in small particle size under 100 nm. It was proven that cavitation energy from ultrasonication was sufficient to produce starch nanoparticle with high starch suspension concentration (10% w/v). SEM observation revealed that these particles have a spherical shape, and the size of starch nanoparticles was in the range of 100-1000 nm.***

Keywords: Sonication, Nanoparticle, Starch, Amplitude, Size distribution

1. Introduction

Bioactive components, such as vitamins, antimicrobials, antioxidants, flavor compounds, dyes, and preservatives, are needed in the food industry. In the industry, these bioactive components are used in the form of integrated food products. The differences of molecular and physical characteristics between bioactive components and food products, such as polarity (polar and non-polar), molecular weight (small or large), physical form (solid, liquid, gas), encouraged micro and nanotechnology (microtechnology and nanotechnology) development. The technology can be a form of encapsulation of bioactive components to produce nano or microcapsules, as well as the production of nanomaterials suitable for food products [1]. Nanoparticles have a large surface area, so they are more active trapping and potential as a "delivery" to bioactive food components (delivery system)[2].

A biopolymer that can be used as raw material for nanoparticles is starch. Starch has nano blocklets that are naturally present in starch granules in their crystalline regions, predominantly composed of amylopectin [3]. The nanometer-sized blocklets can be extracted or isolated with various techniques. In previous, some techniques have been developed to produce starch nanoparticles, such as acid hydrolysis [4], enzymatic hydrolysis [5], high-pressure homogenization [6], gamma irradiation [7,8], ultrasonication [9] and oxidation combined with ultrasonication [10].

In commercial conversions, usually, sodium or calcium hypochlorite is used as the oxidizing agent [11]. Although those oxidation reactions are chemically efficient, they lead to the formation of large amounts of inorganic wastes such as chlorinated products [12]. With an environmental concern as a priority, the oxidant hydrogen peroxide has drawn researchers' great interest because of its low cost and green water decomposition product, water. Many works studies have conducted starch oxidation using hydrogen peroxide [13–15].

Ultrasound energy can be transferred to starch dissolved in aqueous solution through a process called cavitation, which refers to the formation, growth, and violent collapse of cavities in water. The energy provided by cavitation approximately 10–100 kJ/mol, which is within the energy level of hydrogen bonds [16,17,18] was the first to report that starch ultrasound treatment caused physical degradation of the starch granules. Ultrasonication is particularly effective in breaking up the aggregates of nanoparticles formed through hydrogen bonds, reducing the size and polydispersity of nanoparticles [19]. In order to retard the aggregation or to dissociate the nanoparticles, ultrasonic treatments were applied to the starch dispersions [20].

From a critical review of the published literature, studies on the application of sonication applied for SNP production, either sonication itself on its own or combined with other treatment, did not prepare with different starch concentration and hydrogen peroxide additions scarce. In addition, low temperature of sonication to generate SNP production has been reported where an ultrasonic bath was used in water bath sonication yet not in probe sonication [19,21]. Sonication probe for SNP production at room temperature and above applied at room temperature or higher [20].

In this work, oxidation using hydrogen peroxide combined with simultaneous sonication probe treatment at low temperature was used to generate for SNP's formation. Factors such as temperature, starch concentration, vibration ultrasonic power amplitude, and sonication time, are investigated for their influence on the particle size of SNPs. Analysis of morphology and crystalline structure of SNPs was also described to study.

2. Experimental Section

Materials

Commercial corn starch produced by Zhucheng Xingmao Corn Developing Co., Ltd containing starch 91.24% (db). The starch consists of amylose 37.56% (db) and amylopectin 53.67% (db) from total starch. The amylose content was determined by the colorimetric method[22]. Hydrogen peroxide was purchased from Sigma-Aldrich in 30% wt H_2O_2. All reagents were analytical grade, and deionized water was used for sample preparation and analyses.

Starch nanoparticles obtained by sonication and oxidation

Before the sonication process was carried out, a suspension of native corn starch was prepared. Corn starch with a certain concentration (1%, 5%, and 10%) was given the addition of 1% H_2O_2 and water. Then, the starch suspension was given sonication treatment with variations in power amplitude (20%, 35%, 50%), temperature variations (10^{o}C and 30^{o}C), time variations (1 hour and 3 hours). After given sonication treatment, the starch suspension was dried using an oven at 50^{o}C for 24 hours to produce dry starch. The starch then ground to produce starch nanoparticles.

3. Characterization

Size distribution using dynamic light scattering (DLS)

A size distribution determination used Dynamic Laser scattering, a Malvern Zeta sizer Nano (Malvern Instruments Ltd., UK)[24] with slight modification. The starch sample (1%, w/v) was suspended in aqueous solution without filtering. However, the samples centrifuged two times, first was 6000 rpm for 30 min then these were continued at 4000 rpm for 20 minutes. The refractive index of the dispersion phase of the particle used was set as 1.3. Measurements were taken at 25°C and at a 90 Scattering angle.

Particle morphology using Scanning Electron Microscopy (SEM)

The morphology of corn starch nanoparticles examined using a Scanning Electron Microscope (SEM S-4800, Hitachi, Japan). The accelerating voltage was 10 kV. The sample was dissolved in a copper container coated with carbon. Then the copper container put into a freeze-dryer. The dried samples then observed using SEM [19].

4. Results and Discussion

The size distribution of control

The size distribution of corn starch particles was observed using Dynamic Light Scattering (DLS). Starch with more than one peak indicates that the particle size distribution extends, or the particle size is inhomogeneous. This study used two controls. The control of H_2O_2 was prepared without ultrasound (blank H-T10t1h), and control of ultrasound was prepared without H_2O_2 treatment (blank S-P50T10t1h). Both controls were prepared at 10C for the 1-hour process. The size distribution of controls is shown in Fig. 1.

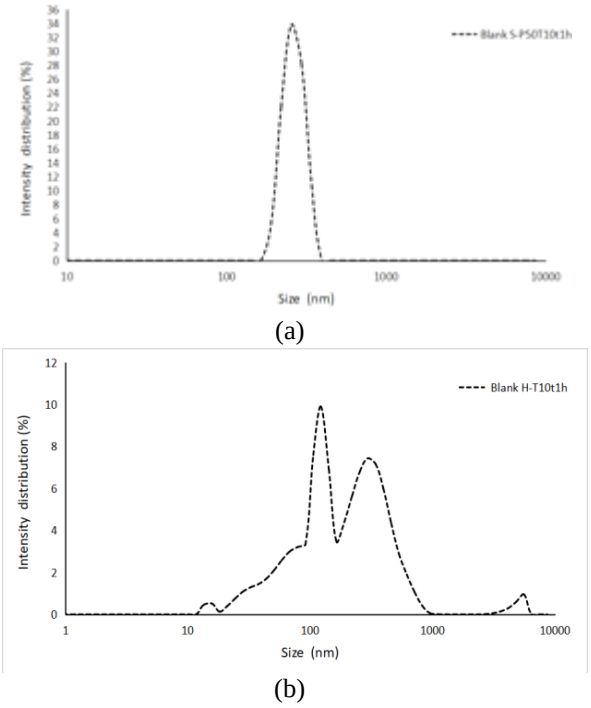


Figure 1: The size distribution of control of H_2O_2 prepared without ultrasound (Blank H-T10t1h),and control of ultrasound prepared without H_2O_2 treatment (Blank S-P50T10t1h).

The reason for combining H_2O_2 and ultrasonication is because we expect to have a small homogenous particle (10-100 nm). Starch with sonication treatment using 50% power amplitude at 10^{o}C for 1 hour produced homogeneous particle size in range 100-1000 nm. On the other hand, the H_2O_2 treatment of starch produced particles in sizes ranging from 10 to100 nm, but the distribution was not homogeneous. By using a combination of both (H_2O_2 and sonication), it was expected to obtain starch particles in the range of 10-100 nm with the homogeneous distribution. [25] argued that an oxidizing agent (H_2O_2) attaching starch granule by biting the granules. It was expected that cavitation energy during the sonication process attacked the starch granules that have been oxidized so that the size reduction process and degradation of starch polymers occurred faster and produced more homogeneous starch nanoparticles. The sonication process can cause disintegration and prevent the formation of aggregates of nanoparticle starch [26]. According to [19], when the sonication process was carried out after the oxidation process, thestarch granule degraded easily into nanoparticle size. In this study, the oxidation-sonication process applied with several process parameters, including power amplitude, time process, temperature, and starch concentration.

The size distribution of starch particle prepared with the different power amplitude

The purpose of this step is to have information optimal power amplitude to produced starch nanoparticle via oxidation-sonication technique. The intensity distribution and average size of nanoparticle prepared with amplitude power 20%, 35%, and 50% for 1 hour oxidation-sonication process at temperature 10°C are presented in Fig. 2.

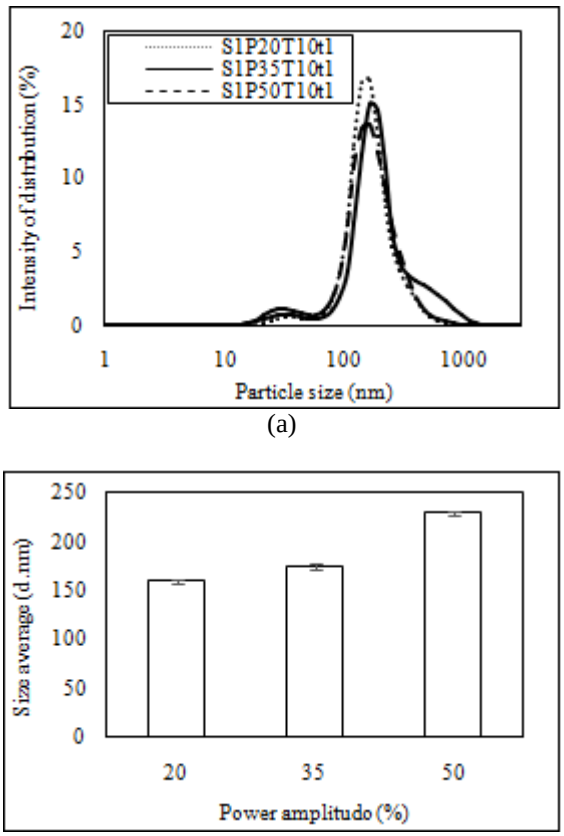


Figure 2: The size distribution (a) and size average (b) of starch nanoparticle prepared by oxidation-sonication with power amplitudo 20%, 35%, 50% at temperature 10°C for 1 hour.

The result in fig. 2. showed that sonication has a bigger impact on the particle size compared to the oxidation effect. The curve of intensity distribution tends to range 100-below 1000 nm than it in below 100 nm. This data also proved that the energy of sonication is bigger than the oxidation reaction. Ultrasonication was very effective in breaking down aggregates of nanoparticles formed through hydrogen bonds, thereby reducing the size and nanoparticle dispersion [27]. The result in this study close to[27], in which the particle size of starch with sonication treatment ranged from 550 to 750 nm.Fig. 2. shows that the intensity of the distribution of starch nanoparticles, the higher power amplitude, the bigger the particle size. We assume that due to the presence of oxidizing agent activity during the oxidation-sonication process, sonication with low power is enough to produce starch nanoparticle. While sonication with high power amplitude triggers starch nanoparticles to aggregate, so the particle size becomes larger. As stated by [27], excessive ultrasound energy produces the opposite effect on particle size distribution, which was an increase in the size of nanoparticles. To add, [28]also revealed that the increase in amplitude triggers the size of the bubble to expand so that the available energy was unable to break down the aggregate.Although amplitude 35% and 50% of oxidation-sonication resulted in bigger particle size, its size was still under 1000 nm.

The size distribution of starch particle prepared with the different processing time

The purpose of this step is to have information optimal processing time to produced starch nanoparticle via oxidation-sonication technique. The size distribution of nanoparticle prepared with amplitude power 20%, 35%, and 50% for 1 hour and 3 hours oxidation-sonication process at temperature 10°C are presented in Fig.3.

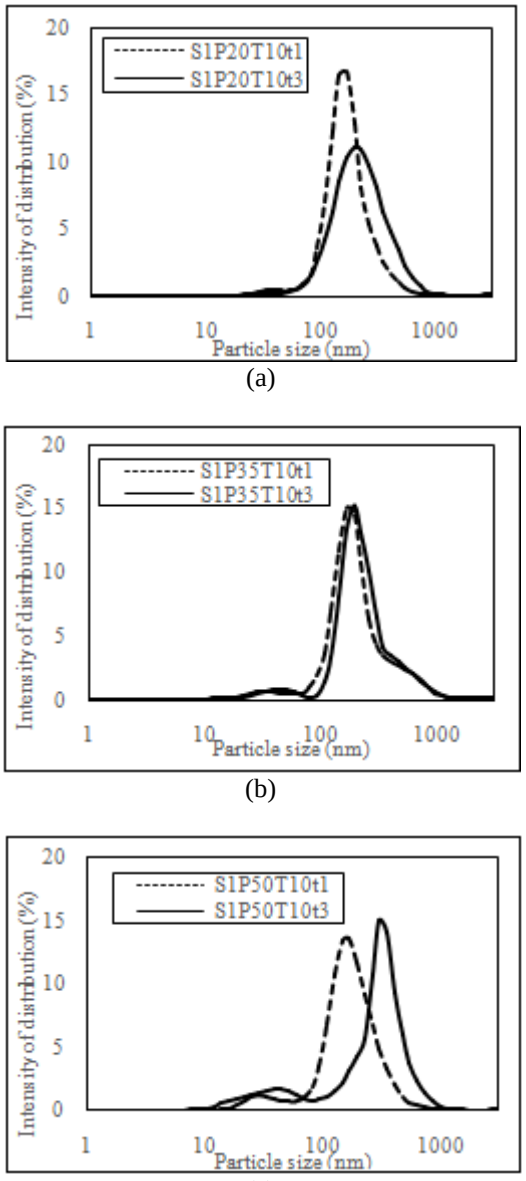


Figure 3: The size distribution of starch nanoparticle prepared by the oxidation-sonication process with power amplitude 20% (a), 35% (b), 50% (c) at 10°C for 1 and 3 hours.

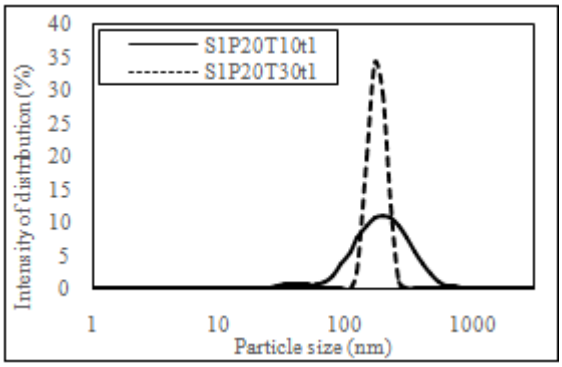
Fig. 3. shows the longer processing time the curve was shifted to a bigger particle size. The longer time provides more opportunity for free radical of hydrogen peroxide to attach small particle which has been formed previously. [13] found that H_2O_2 produced the oxidizing species, such as free radicals and nascent oxygen. We assume the free radical

attached to nanoparticle promoted the nanoparticle close to each other and tends to aggregate, forming bigger particle size. Therefore, during the 3 hours oxidation-sonication, some big particle size (almost 1 Mikron) was detected, especially in the 3 hours of high power (amplitude 50%) process (Fig 3.c). This result was in line with previous work by [27]. They found that excessive ultrasonication time resulted in the opposite effect on particle size distribution. In their study, the particle size increased when ultrasonication extended from 80 minutes to 120 minutes. Another work by [21]showed that starch nanoparticles were formed during 75 minutes of ultrasonic treatment.

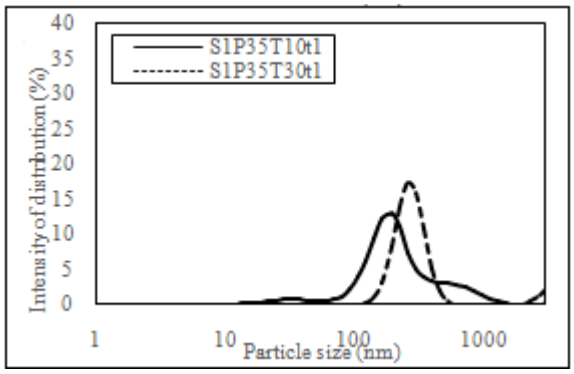
Fig. 3. presents that the higher the power amplitude, the more the curve of size distribution shifted to a bigger particle size area. [28] revealed that the increase in amplitude triggers the bubble's size to expand. We suggest the higher power amplitude, the bigger the bubble was produced. Therefore, from 20% (Fig 3.a), 35% (Fig 3.b), then 50% (Fig 3.c) of power amplitude, the shifting of the curve was more. The long run of the oxidation-sonication process means more time for big bubbles to push small particles closer to each other and form a bigger particle size. Although three hours of oxidation-sonication resulted in a bigger particle size, its size was still under 1000 nm.

The size distribution of starch particle prepared with different temperature

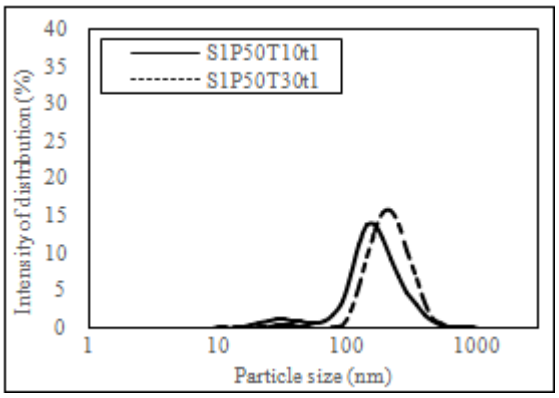
The purpose of this step is to have the information on the oxidation-sonication temperature to produce starch nanoparticle. The size distribution of nanoparticle prepared with amplitude power 50% for 1h oxidation-sonication process at temperature 10°C and 30°C are presented in Fig.4.



(a)



(b)



(c)

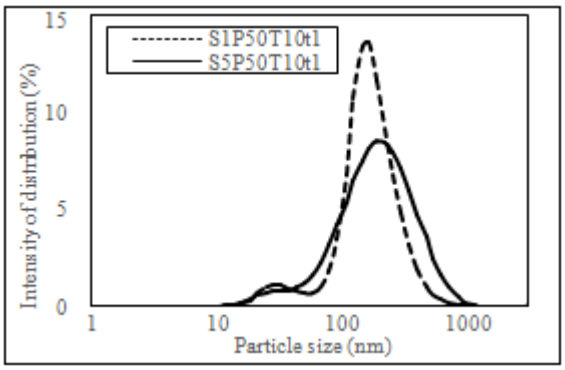
Figure 4: The size distribution of starch nanoparticle prepared by oxidation-sonication process at temperature 10°C and 30°C with power amplitude 20% (a), 35% (b) and 50% (c) for 1 hour.

Fig. 4. shows that for preparing starch nanoparticle in size below 100 nm, oxidation-sonication conducted at 10°C produced a smaller particle than it ran at 30°C. The size distribution curve was shifted to the left side at all power amplitude conditions. According to [29], the sonication process's temperature affected water vapor pressure. Therefore, the higher temperatures could reduce transmitted energy, resulting in a reduction of the cavitation intensity. In this study, lower cavitation energy resulted in a bigger particle size. In line with the graphic of Fig. 2, Fig. 4. also shows that the higher amplitude, the bigger particle size. Therefore, the gap between the two curves of different temperature process was smaller in amplitude 50% compared to the curve gap of amplitude 20% and 35%, especially in the size area below 100 nm.

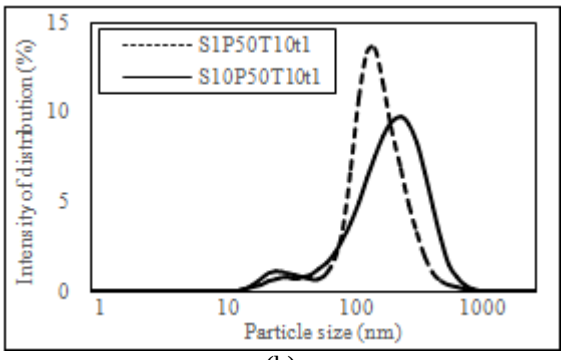
In addition, low temperatures during the sonication process caused water molecules did not diffuse into the amylopectin chain, so the plasticization phase of amylopectin does not occur [21]. We assume no plasticization phase caused low density in the starch suspension, so H_2O_2 and the bubble of cavitation energy were moved easier and resulted in small particle size under 100 nm. Although the temperature 30°C of oxidation-sonication resulted in a bigger particle size, its size was still dominated by under 1000 nm.

The size distribution of starch particle prepared with the different starch concentration

The purpose of this step is to have the information on starch suspension concentration for preparing starch nanoparticle prepared via the oxidation-sonication technique. The size distribution of starch nanoparticle prepared with starch suspension 1%, 5%, and 10% for 1hour oxidation-sonication process at temperature 10°C are presented in Fig.5.



(a)



(b)

Figure 5: The size distribution of starch nanoparticle prepared with 1%, 5%, and 10% starch suspension treated by oxidation-sonication sonication with amplitude 50% temperature 10°C for 1 hour.

Fig.5. shows that the size distribution curve ofstarch nanoparticle prepared by 5% and 10% starch suspensiontends shifted to the area of bigger particle size. According to [27], particle size would increase as the starch concentration increased. We assume in the higher starch suspension concentration, the movement of H_2O_2, and the bubble of cavitation energy was less than in the lower starch concentration. As we know, the equation of mass density is $\rho = m(g)/V(cm^3)$, so it means the higher starch concentration in the same water volume, the suspension will be denser. Interestingly, after a 1-hour oxidation-sonication process, 5% and 10% starch suspension formed starch nanoparticles in the range of 100-1000 nm, like 1% starch suspension (Fig. 5). It was proven that cavitation energy was sufficient to produce starch nanoparticle with high starch concentration. According to [19], sonication (for 3 hours) was effective to degrade the oxidized starch (the oxidizing agent was NaOClcatalyzed by 2,2,6,6-Tetramethylpiperidin-1-yl) oxyl (TEMPO)) into nanoparticles. The difference is that in this work, the oxidizing agent was H_2O_2, and the process was conducted for 1h.

The morphology of native starch and starch nanoparticles

The purpose of this step is to have information on the morphology of native corn starch granule and starch nanoparticles prepared by the oxidation-sonication method. Fig. 6. presents the FE-SEM observation result of native corn starch and starch nanoparticles. The starch nanoparticles in Fig. 6. are starch nanoparticles prepared by 20% amplitude power for 3 hours process, 50% amplitude power for a 1hour process, and starch nanoparticle prepared by 5% starch suspension. All processes of those starch nanoparticles were via oxidation-sonication at temperature 10°C.

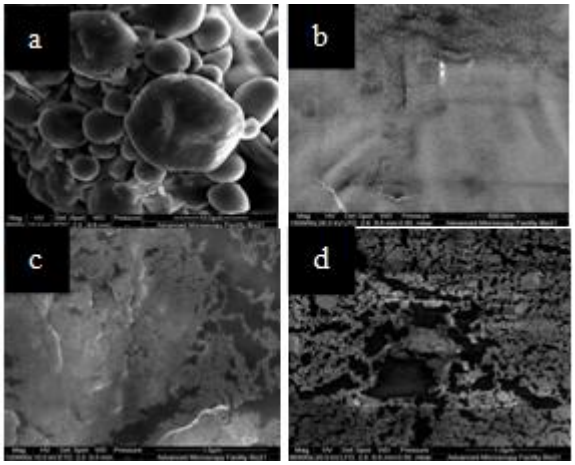


Figure 6: FE-SEM of native corn starch and corn starch nanoparticles. (a) Native corn starch. (b) corn starch nanoparticles prepared with 1% starch suspension, 1% H_2O_2, and sonication at 20% amplitude for 3 hours. (c) corn starch nanoparticles made with 1% starch suspension, 1% H_2O_2, and sonication at 50% amplitude for 1 hour. (d) corn starch nanoparticles prepared by 5% starch suspension, 1% H_2O_2, and sonication at 50% amplitude for 1 hour. Scale bar for (a) is 10 µm, (b) is 1 500 nm, then (c) and (d) are 1 µm.

FE-SEM obtained the morphology of starch nanoparticle. Electron microscopic observation verified that sonication treatment produced nanoparticles. Fig. 6. shows that the native corn starch was ellipsoid with a diameter size of around 5-15 µm. This result was similar to previous work by [19]. After oxidation-sonication in varied process parameters, the starch particle size was much smaller with more than 1000 times size reduction.The starch nanoparticle particle size was estimated at 100-1000 nm (Fig 1-5). This result closed to [29] work result that the nanoparticle showed the size of starch nanoparticles around 109-1050 nm. However, the corn starch nanoparticle in this work was a slight difference with the corn starch nanoparticle produced by similar combination method oxidation and sonication in previous work by [19]. The differences in particle size could be caused by crop resource, the arrangement of starch component, and preparation method [30–32]. Regarding the preparation method, previous work applied an oxidizing agent NaOCl and catalyzed by TEMPO [19], but in this study, the oxidizing agent was H_2O_2 without catalysator. The spherical shape of starch nanoparticles may be related to starch blocklets presented in starch granules [33].

5. Conclusions

A combined oxidation process involving ultrasonic and hydrogen peroxide could be applied as an alternative technique to prepare starch nanoparticle. At low temperature, H_2O_2 and the bubble of cavitation energy were moved easier and resulted in small particle size under 100 nm. Also, it was proven that cavitation energy from ultrasonication was sufficient to produce starch nanoparticle with high starch suspension concentration (10% w/v). Although the higher amplitude formed bigger particle size,

the starch nanoparticles showed size in range 100-1000 nm with the spherical shape.

6. Acknowledgments

We are grateful for financial support from the School of Chemistry, Faculty of Science, the University of Melbourne for providing the opportunity as an academic research visitor at the Sonochemistry Laboratory [visitor ID 123162]. Also, thankful to the Indonesian Ministry of Research, Technology and Higher Education through doctoral study scholarships [grant number 1420 / E4.4 / 2014], doctoral dissertation research grants [grant number 067 / SP2H / LT / DRPM / IV / 2007] and sandwich scholarship programs [grant number 1597 / D3.2 / PG / 2017].

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