Preparation and characterization of sago (metroxylon sp.) Starch nanoparticles using hydrolysis-precipitation method

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Abstract. At present, the potential for sago starch has not optimally been utilized. The characteristic of this starch is the main problem in using it as industrial raw material. One of efforts to increase the added value of starch is to modify it in the form of starch nanoparticles. Therefore, finding the best and cheapest practice to produce it will a big advantage. One way to do it is by modifying the technique that has already existed. This research is aiming, first to find the best and optimal technique to produce sago starch nanoparticles by combining hydrolysis with precipitation technique and second, calculating the yield and characteristics of sago starch nanoparticles produced from this method. The result showed that hydrolysis–precipitation combination method has succeeded to produce sago starch nanoparticles. The optimum variable was 12 hours hydrolysis, precipitation by ethanol, with produced 25% yield and 21.98 nm particles size. Type of nano particle that is produced is amorf with 41% crystalline. Starch nanoparticle is utilized for reinforcing bioplastic. Eventhough, the results show a potential technique on producing sago starch nanoparticles, but there are still a lot to be done to find the best way to produce it in mass production.

1. Introduction
Indonesia has around 50% of world sago production. The potential for sago production in Indonesia is estimated as much as 2 million tons per year. The use of starch in industry is very broad which not only in the food but also in non-food fields. At present, the potential for sago starch has not optimally been utilized. The natural starch properties are difficult to be used as industrial raw material. Therefore, starch needs to be modified to expand the application. Lee et. al. stated that the development of nanotechnology at the present time has impact on increasing demand of small-sized particle raw material for industries [1].

Nanotechnology offers various opportunities for the development of innovative and applicable products in various fields such as packaging, biomedicine, electronics, optics and agriculture. New unique properties will emerge if the material is in the nanoscale because of changes in the functional properties of materials, and changes in the nature of dispersion. These new properties are not found in large-sized materials. Changes in the functional properties of nanoparticles can create competitive products. This is the demand of users who want to use nanotechnology applied in the various fields.
Recently, the nanoparticle starch products used for industry are BioTRED and Eco-sphere TM. BioTRED (Novamont, Italy) is developed in the tire industry which collaborate with American companies (Goodyear, USA). Starch nanoparticles replace parts of black carbon and silica in tires in order to improve the quality of tire. Eco-sphere TM is an starch-based biolatex as substitute of oil-based latex used for oil-based coatings and binders for paper and paperboard. Research on starch-based nanoparticles is still open, such as; to explore the type of starch that is suitable for nanoparticles including the preparation method. Mastery of nanotechnology will enable various new discoveries that provide added value to a product, even creating value for a product. Nano-sized starch preparation is easy because starch granules are inherently composed of nano crystalline blocklets [2], so that they can form nanoparticles spontaneously (self assembly).

Starch granules consist of amorphous and semi-crystalline layers arranged intermittently in leukoplast. Amylose and amylpectin molecules arrange starch granules in a certain pattern [3]. In its original form, starch is small grains called starch granules. Starch granules have distinctive size, shape, uniformity and shape of the hilum that varies depending on the type of starch, so that it can be used to identify the type of starch. In granules, mixtures of molecule structures are linear and branched, which arranged radially in concentric cells and forming rings and lamellae. The formation of lamella in starch is thought to be a result of the molecular coating on the granule, while the hilum is the point where granules begin to develop. The structure of starch can be seen in Figure 1.

In general, there are three ways in making nanoparticle starch which are acid or enzymatic hydrolysis, regeneration (precipitation) and mechanical treatment. The acid hydrolysis process is carried out by using strong acids to form crystalline starch. The strong acids degrade amorphous regions in starch granules. Starch formed from the results of acid hydrolysis is crystalline starch which has different characteristics from its natural starch. The different characteristics of crystalline starch can also be influenced by time leverage of acid hydrolysis. Ma et. al. suggested that nano-sized particles from starch can be produced by precipitation treatment using organic solvents such as ethanol, butanol, and acetone. Starch formed from precipitation using different solvents will produce different characteristics [5].

Hydrolysis is done by strong acids with low concentration. Regeneration methods are produced through precipitation of starch dispersed with organic solvents [6]; [5]; [7] which produce starch nanoparticles. The third method of nanoparticle production is mechanical and thermal treatment with high pressure homogenisation and microfluidizers [8]; a combination of high-pressure homogenization techniques with crosslinking [9], and production with extrusion processes [10]. Nanocrystal starch are
produced in hydrolysis of starch using HCl and H$_2$SO$_4$ at 35-45°C. Starch nanoparticles can also be produced from precipitation of complex formation among amylose fractions with hydrophobic components such as n-butanol [6]; [11] atau ethanol [5]; [12].

Some weaknesses of the previous starch nanoparticle preparation methods were low yields, long processing times, and quite expensive equipment. This causes the availability of nanoparticle starch is still rare in the market and the price is quite high. Modified the method to supply nanoparticles with low production costs and high yields is a quite interesting challenge. One of the ways to overcome this is to do the modified preparation of nanoparticle starch. Research on developing an efficient method for the supply of nanoparticle starch on a large-scale industry is still very much needed. This research tried to combine hydrolysis and precipitation methods in producing nanoparticle starch. The purpose of this research was first, to obtain the optimal production process in making sago starch nanoparticles; second, to know the yield and characteristics of sago starch nanoparticles produced from this method.

2. Materials and Methods

2.1 Materials
Materials used in this research were sago starch (Metroxylon sp.), which produced from Koto Marapak area, District of Pariaman, West Sumatera, aquadest, ethanol 95%, NaOH, HCl 2.2 N, n-butanol, and filter papers.

2.2 Equipments
Equipment used are magnetic hotplate stirrer, oven, glassware, analytical scales, spray dryer Buchi B-290, vacuum pump, refrigerator, packaging and thermometer. Meanwhile, the equipment used to characterize starch nanoparticle are Particle Size Analyzer (PSA) Malvern MAL1096681, Scanning Electron Microscopy (SEM) Hitachi S-3400N and X-Ray Diffraction (XRD) Xpert Pro Analytical PW30 / 40.

2.3 Methodology
This research will be conducted in 4 stages. Stage 1 is the characterization of raw materials. Characterization includes physical, chemical and functional characteristics. Stage 2 is the hydrolysis of starch. Starch is made suspension in 2.2 N HCl solution with a ratio of 1: 2. Then the suspension of starch was incubated in a shaker bath at 35°C for 12, 24, and 48 hours. The starch suspension which has hydrolysis treatment is then neutralized with 1 N NaOH, then washed with ethanol and distilled water. Washed starch is then dried in an oven at 40°C for 24 hours until it reaches a moisture content of 10-12%. Stage 3 is precipitation of starch. The precipitation method was used in this study is precipitation using ethanol which is adapted from Ma et al. (2008) [5]. Starch was dissolved in distilled water with a ratio of 1:15, was heated to perfect gelatinization. After perfect gelatinization, ethanol (butanol) is added by dropping it slowly while stirring rapidly with a magnetic stirrer, then was cooled. The precipitate formed was separated by centrifugation and dried with a spary dryer. Stage 4 is the calculation of yield and characterization of sago starch nanoparticles. Characterization includes Particle Size Analyzer (PSA), Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD).

2.4 Particle Size Analyzer (PSA) tests
Characterization was carried out using the PSA brand Malvern Serial Number: MAL1096681. This characterization was carried out to determine the particle size distribution of sago starch nanoparticles. The steps taken in the analysis using the PSA are as follows: 1. The sample is put into a dispersant in the form of distilled water pH 7 and then placed in a cuvette of 3 mL. 2. The cuvette then passes through visible light so that diffraction occurs. 3. Measurement of particle size utilizing the principle of visible light scattering, the output produced is a graph of particle size distribution.
2.5 Scanning Electron Microscopy (SEM) tests

Testing using SEM Hitachi 3400 series N. The use of this SEM can also determine the surface morphology and particle size of sago starch. The steps in this SEM test are as follows: 1. The sample to be analyzed is prepared and glued to the specimen holder (dolite, double sticky tape). 2. Samples that have been installed on the holder are then cleaned with a hand blower. 3. Samples are included in the coating machine to be given a thin layer of gold-palladium for 4 minutes to produce a layer with a thickness of 200-400 Å. 4. Samples are included in the specimen chamber. 5. Observation and shooting at the SEM layer by adjusting the desired magnification. 6. Spot determination for analysis on the SEM layer. 7. Shooting SEM images.

2.6 X-Ray Diffraction (XRD) tests

XRD characterization aims to determine the crystal system. X-ray diffraction methods can explain the lattice parameters, the type of structure, the arrangement of different atoms in the crystal, the presence of crystal imperfections, orientation, grain and grain size [13]. This technique is used to identify the crystalline phase in the material by determining the parameters of the lattice structure and to get the particle size. The formula for calculating the crystallinity index from the sample using the Segal method is as follows:

\[ CrI = \left( \frac{I_{002} - I_{AM}}{I_{002}} \right) \times 100 \]  

\[ CrI = \text{The degree of relative crystallinity} \]
\[ I_{002} = \text{Maximum intensity of diffraction pattern 0 0 2} \]
\[ I_{AM} = \text{The intensity of diffraction in the same unit at 12-18°} \]

3. Results and Discussion

3.1. Characteristics of Sago Starch

The results of chemical, physical and functional characterization of sago starch (Metroxylon sp.) can be seen in Table 1. The quality standards of sago starch in Indonesia are listed in the Indonesian National Standard (SNI 3729: 2008).

| Table 1. Chemical, physical and functional characteristics of sago starch |
|---------------------------------------------------------------|
| **Parameter**       | **Sago** | **SNI 3729:2008** |
| Chemical:           |          |                  |
| Water content (%)   | 13.75    | Max 13           |
| Ash content (%)     | 0.39     | Max 0.5          |
| Fiber content (%)   | 1.03     | Max 0.5          |
| Starch content (%)  | 85.16    | Min 65           |
| • Amylose (%)       | 28.93    | -                |
| • Amylopectin (%)   | 71.07    | -                |
| Physical:           |          |                  |
| Form                | Elips    | -                |
| Granule size (mm)   | 57.56    | -                |
| Fracitcon passed 100 mesh (%) | 68.65 | -                |
| Gelatinization temperature (° C) | 85 | -                |
| White degree (%)    | 71.22    | -                |
| Functional:         |          |                  |
| Solubility (%)      | 5.36     | -                |
| Water absorption (%)| 60.38    | -                |
Starch is a glucose polymer with α-glycosidic bonds arranged into amylose and amylopectin and also contains other ingredients such as lipids and proteins. Amylose and amylopectin components contained in starch granules have different ratios, in which the amylopectin component has higher ratio than amylose. These two components are one of the factors that can influence differences in physical, chemical and functional characteristics of starch. Straight amylose structures tend to be in the amorphous part of starch granules. Meanwhile, amylopectin which can form double helical structure is responsible for the crystalline part of starch granules.

Based on the results of the characterization, the water content of the sago starch that will be used have not meet the specified quality standards so that further drying is needed to obtain water content below 13%. Crude fiber content is another minor component found in starch. This crude fiber consists of cellulose, hemicellulose, and lignin. The type of sago starch used has a high fiber content according to SNI 3729: 2008.

3.2. Starch Nanoparticles by Hydrolysis-Precipitation Method

The nanoparticle starch in this research included nanocrystalline starch and nanoparticle starch. The method used for the production of nanoparticle starch is the combination of hydrolysis-precipitation, so the final product is called nanoparticle starch. Nanoparticle starch is a starch that has gone through modification process in the structure and size of starch. The preparation of nanoparticle starch in this research was carried out in 2 process stages; lintnerization (slow acid hydrolysis) and precipitation. The lintnerization treatment was carried out in order to degrade the long-chain amylose fraction and branching points in amylopectin found in the amorphous area so that a short chain amylose fraction was produced with a lower molecular weight. Lehmann et. al. [14] suggested that if the short chain amylose fraction in starch increases, there will be more crystallized amylose fraction because the amylose fraction as a linear structure will facilitate cross-linking with hydrogen bonds so that the amylose structure forms a compact crystallite. By implementing this lintnerization process, it is expected that there will be the crystallinity increase of starch.

The lintnerization process was done by using strong acids in lower temperature of starch gelatinization. Strong acids can hydrolyze glycosidic bonds to produce shorter chains of amylose and lower molecular weight. In this process, there are two stages of attack that occur in starch granules, which are the rapid attack stage on the amorphous area and the slower attack stage on the amylopectin fraction found in the crystalline area [15].

Lintnerization time can affect the degradation rate of amorphous areas during the hydrolysis process. Longer time of the operation is thought to increase the destruction rate of the amorphous areas by acid, and it can even be said that the acid will degrade the structure of amylopectin in the crystalline area that can be reached by acids. The integration process causes destruction of the amorphous region in starch granules. Amorphous areas are more easily affected by chemical reactions than crystalline areas because hydrogen bonds in the amorphous areas are weaker than those in crystalline areas. The long chain amylose found in the amorphous area is cut off by acid to be simple sugar. According to Wulanandi et al. [16], the lintnerization process does not only terminate the linear chains of amylose and amylopectin branching found in the amorphous areas, but also terminate the amylopectin chains that are reached by acid in the crystalline areas. The hydrolysis process occurs slowly, the attack on the amylopectin fraction found in the crystalline areas.

The lintnerization process only changes the chemical structure of starch, but this process has not been able to produce nanoparticle starches yet, so that further processing is needed to obtain smaller particles. Ma et. al. [5] suggested that nanoparticle starch can be synthesized by precipitation of starch solution using organic solvents. The types of organic solvents used are 95% ethanol and n-butanol. The formation of nanoparticle starch occurred when starch received high temperature treatment during the gelatinization process. With the thermal and mechanical energy given to the starch during heating above its gelatinization temperature, there is destruction of covalent and hydrogen bonds in the structure of double helix amylopectin and melting of the crystallite portion that result smaller size of starch particle.
By doing damage to these bonds, starch granules will easier to form smaller particles, including Nano size particle.

3.3. Characteristics of Starch Nanoparticles

3.3.1 Particle Size Analyzer (PSA). Starch has various shape and size of granules depending on its source type. Based on the characterization of raw materials, sago starch has an average size of 57.56 μm. PSA test results can be seen in Table 2. The PSA test results showed that there had been size reduction of the starch particles in the hydrolysis stage. It is followed by the precipitation stage which had also succeeded in reducing the size of the starch to obtain nanoparticle starch. The test results showed that the hydrolysis-precipitation method could reduce the size of sago starch into nanoparticles. In general, ethanol and butanol solvents can be used as precipitates. The use of ethanol is more economical for large-scale production. The particle size produced in this research was smaller than the nanoparticle starch using precipitation method which conducted by Chin et. al. [12].

| Sample                  | Size particle (nm) | Yield (%) |
|-------------------------|--------------------|-----------|
| Acid hydrolysis:        |                    |           |
| 12 hours                | 789.30             | 80        |
| 24 hours                | 741.00             | 74        |
| 48 hours                | 738.10             | 72        |
| Acid Hydrolysis-precipitation: |                |           |
| 12 hours, ethanol       | 21.98              | 25        |
| 24 hours, ethanol       | 87.69              | 23        |
| 48 hours, ethanol       | 97.50              | 20        |
| 12 hours, n-butanol     | 7.57               | 23        |
| 24 hours, n-butanol     | 25.50              | 23        |
| 48 hours, n-butanol     | 178.70             | 22        |

Nanoparticle production using the complex precipitation formation method is done based on amylose properties which can form complex inclusions with hydrophobic components such as ethanol and n-butanol. When amylose accommodates the hydrophobic molecule, a single left helical structure will be formed. The crystal is formed as a result of retrogradation or rearrangement of starch structure that has passed through gelatinization.

3.3.2 Scanning Electron Microscopy (SEM) Analysis. Morphology of starch granules can be seen using SEM test. SEM test results of starch formed during the process of hydrolysis-precipitation can be seen in Fig 2. At hydrolysis stage, particle size has been reduced from its natural starch with size of 57 μm into 0.7 μm. Sago starch which has done hydrolysis process still has the same shape as its natural starch as shown in Fig. 2 (a), (b), (c). This happens because strong acids are able to hydrolyze glycosidic bonds to produce amylose with shorter chains and lower molecular weight. The linterization process plays a role in the destruction of the amorphous area found in starch granules and reduces the size of starch.
Figure 2. Test results of SEM (a) 12 hours of hydrolysis, (b) 24 hours of hydrolysis, (c) 48 hours of hydrolysis, (d) 12 hours of hydrolysis – ethanol precipitation, (e) 24 hours of hydrolysis – ethanol precipitation, (f) 48 hours of hydrolysis – ethanol precipitation, (g) 12 hours of hydrolysis – butanol precipitation, (h) 24 hours of hydrolysis – butanol precipitation, (i) 48 hours of hydrolysis – butanol precipitation

Figure 2 (d) - (i) shows that the precipitation process affects the particle size and its morphology. The size of starch is smaller than that of hydrolysis (linterization) result. As explained earlier, the presence of heat and mechanical treatment during the precipitation process can cause the formation of smaller particles when starch is degraded. This mechanical treatment causes the intermolecular bonds of amylase and amylopectin to be cut off when starch is retrograded so that the shape and size of starch particles do not return to their original form. Based on the analysis of starch morphology using SEM, it can be seen that the starch particles after homogenization are still not completely separated and still fused to form lumps. This happens because the starch nanoparticles have many hydrogen bonds on its surface so it tends to merge among the particles.

3.3.3 X-Ray Diffraction (XRD) Analysis. The calculation results of crystallinity index using the Segal formula based on XRD testing can be seen in Table 3. Hydrolysis time can affect the degree of starch crystallinity. The crystallinity of starch has increased from its native starch after being hydrolyzed. In this case, the hydrolysis process does not change the pattern of starch crystallinity, but only changes the index of its crystallinity. This shows that the hydrolysis process only causes destruction of the amorphous area to obtain more crystalline starch and does not make structural changes.
Table 3. Index Crystallinity of Sago Starch Nanoparticles

| Sample                        | Index Crystallinity (%) |
|-------------------------------|-------------------------|
| Native sago starch            | 39                      |
| Acid hydrolysis:              |                         |
| 12 hours                      | 54                      |
| 24 hours                      | 58                      |
| 48 hours                      | 59                      |
| Acid hydrolysis - precipitation: |                      |
| 12 hours, etanol              | 41                      |
| 24 hours, etanol              | 36                      |
| 48 hours, etanol              | 36                      |
| 12 hours, n-butanol           | 34                      |
| 24 hours, n-butanol           | 29                      |
| 48 hours, n-butanol           | 31                      |

The crystallinity of starch tends to decrease from the hydrolysis process to precipitation. The crystallinity pattern in precipitated measurement results of XRD has more sloping pattern. This more sloping pattern has property changes to be amorphous after precipitation. The change of crystallinity pattern of nanoparticle starch is caused by the opening of double helix structure in the crystalline areas. In addition, the crystallization process of short chain amylose that forms double helix can also be occurred so that it causes crystalline form changes in starch granules and decreases the value of crystallinity index.

3.4 Yield Analysis
The yield of nanoparticle starch produced by the hydrolysis-precipitation method can be seen in Table 2. The yield value in the hydrolysis stage can reach an average of 75%. The decrease in yield along with hydrolysis time is caused by the degradation process of amylose structure and branching of amyllopectin during the linearization process. In treatment time of hydrolysis; 12, 24 and 48 hours, sago starch passes through destruction of the amorphous area which keep increasing along with the length of hydrolysis process. At this stage, more starch structures are cut off into simple sugars which dissolve in the filtrate so that the yield is lower. This can be seen in Table 2, which shows a decrease in yield value occurs along with the increase of hydrolysis time.

The average yield in the final stage of the process reaches 22.7%. This value is quite high compared to several other methods used by previous researchers. Kim & Lim [6] have obtained nanoparticle starch with yield value of 6.78% and Ma et al. [5] have also succeeded to obtain nanoparticle starch with 15% yield using the precipitation method treatment.

Precipitation process is a critical process in the production of starch nanoparticles by the precipitation-hydrolysis method. The yield in the precipitation stage has decreased significantly from the previous hydrolysis stage. This decrease occurs during the gelatinization process. The gelatinization process results in damage to hydrogen bonds which has function to maintain the structure and integrity of starch granules. The amount of amylose-amyllopectin fraction is very influential on the starch gelatinization process. Amylose has a smaller size with non-branching structure. Meanwhile, amyllopectin is a large molecule with many branching structures and forming double helix. When the starch is heated, several double helix amyllopectin fractions are stretched and released when there is hydrogen bond broken. If higher temperature is given, more hydrogen bonds will be cut off and water will be absorbed into the starch granule. In this process, the amylose molecule is released into the water phase that wraps the granule, so the structure of starch granules becomes more open, and more water enters the granule and causes the granule to swell and increase its volume. The water molecule then forms hydrogen bonds with the hydroxyl groups of sugars from the amylose and amyllopectin molecules.
On the outside of granule, the amount of free water decreases, while the amount of amylose released increases. Amylose molecules tend to leave granules because the structures are shorter and soluble.

4. Conclusion
The combined method of hydrolysis-precipitation has succeeded to produce nanoparticle starch of sago. The optimal process for 12 hour hydrolysis and precipitation with ethanol. This process results yield 25% and particle size 21.98 nm. The type of nanoparticle starch produced was amorf with 41% index crystallinity.

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