Effect of Acid And Hydrolysis Duration on The Characteristics of Arrowroot and Taro Starch Nanoparticles

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Abstract. Arrowroot and taro are underutilized tuber. Modification of starch into starch nanoparticles will open to opportunities for further development. The aim of this research was to find out the influence of acid types and hydrolysis length on the characteristics of the resulted starch nanoparticle. There were group of treatments including source of starch: arrowroot and taro beneng starch, and the acid types was HCl and H2SO4, and the length of hydrolysis was 3 and 5 hours. The results showed that the yield of arrowroot nanoparticle starch was higher than taro, 21.64 - 34.44% and 33.83 - 51.22%, respectively. Hydrolysis with HCl and H2SO4 produced starch nanoparticles (NP) with different characteristics. Treatment with HCl provides higher yield than that of H2SO4, but particle size was larger. The average size of the taro and arrowroot starch nanoparticles produced by hydrolysis by H2SO4 was 379.2 nm and 464.4 nm, respectively, with high polydispersity index of 0.480 and 0.621. Meanwhile, the crystallinity of taro and arrowroot starch NP increased from 44.1 into 87.3% and 43.5% into 51.3%, respectively. Acid hydrolysis improved solubility of starch nanoparticles but decreased the swelling power and gelatinization temperature. Starch nanoparticles can be implemented as a reinforcing agent for packaging, and carrier material for phytochemicals or agro-chemicals.

1. Introduction
Indonesia has abundant local starch-source crops which have potential to be developed as industrial materials. Among them are arrowroot and taro tuber as potential starch sources. Starch has been widely used in various industries such as food, textile, and pharmacy. It is used in many applications including surface sizing, as a food emulsifier, fat replacer, excipients for tableting and drug delivery carriers[1].

Starch is mainly composed of amyllose (AM) and amylopectin (AP), AP is expected play dominating role in properties of starch because of its semi crystalline nature which mainly constituted of short branched-chains. Moreover, it has a lot of hydroxyl groups on the backbone. The primary and secondary hydroxyl groups at C-2, C-3, and C-6 of each glucose residue make starch hydrophilic and available for further modification [2]. Preparation into starch nanoparticles is one kind of modification to enhance starch performance [3]. A starch nanoparticle (NP) has benefits in terms of higher surface area, lower viscosity at higher concentration and higher entrapment of active ingredients [4]. Moreover, the micro- and nano scale structure of starch granules influences the biological and the physicochemical properties of food [5]. Particle size of starch granules contributes to specific functional qualities such as texture, volume, consistency, and moisture and shelf stability [6]. Nanoparticles and nanocrystals synthesized from starch exhibit reinforced thermal, higher temperature resistance, and mechanical and...
barrier properties [7] [8]. Because of these unique characteristics, starch nanocrystals and nanoparticles are becoming important for potential applications in food science and medicine sector.

Among the methods to produce starch nanoparticles is using acid hydrolysis. Hydrolysis using mild acid like hydrochloride and sulphuric acid at temperature below gelatinization temperature is often to produce starch nanocrystal. The residue after acid hydrolysis contains the starch nanocrystals which have high crystallinity and nanoscale platelet morphology. Angellier-Coussy et al. investigated the molecular structure of waxy maize starch nanocrystals found that there were two major groups of dextrins in the nanocrystals with average degrees of polymerization of 12.2 and 31.7 [9].

Many research have been done to produce starch NP by acid hydrolysis using several kinds of starch sources such as potato starch, maize starch, rice and other kinds of starch, however, there is very limited information about NP starch from taro and arrowroot starch. The botanical origin of starches which have different properties may produce different characteristics of the resulted acid starch NP. The objective of the research was to find out the influence of acid types and hydrolysis duration on the characteristics of the resulted starch nanoparticle

2. Material and Methods
The raw materials that used were arrowroot starch (Maranta arundinaceae) from Creole varieties, meanwhile taro starch processed from taro beneng (Xanthosoma undipes) tuber, both two kinds of tuber derived from Pandeglang, Banten Province. Processing tubers into starch was carried out at Pilot Plant Laboratory of ICAPRD, Bogor. Chemicals that used for this process among others HCl, ethanol, methanol, and other chemicals used for analysis. The chemicals were analytical grade and used as received.

2.1. Production and Characterization of Cristalline Starch by Acid Hydrolysis
Production of crystalline starch carried out by acid hydrolysis [10] and [11]. The starch (arrowroot and taro starch) was made suspension in HCl 2.2 N and H2SO4 3.16 N with ratio 1:2 and incubated at temperature 40°C for 3 and 5 days using rocking waterbath. After hydrolysis, starch suspension was centrifuge and wash several time by distilled water. Starch was then neutralized by NaOH 1 N until pH 7.0, then homogenised using ultra turrax for 2 minutes at 13,000 rpm. The suspension washed with ethanol and distilled water. The then dried at temperature 40°C for 24 hours.

The research design used was completely random block design with three replications. Parameters observed in starch nanoparticles include: yield, morphology (SEM), particle size distribution (PSA), gelatinization profile (RVA), and crystallinity pattern (XRD).

3. Result and Discussion
The yield of starch nanoparticles on various types of acid and hydrolysis duration on taro and arrowroot starch NP is shown at Tables 1 and 2. From Table 1 it can be seen that the yield of taro starch NP was statistically not significantly different both for H2SO4 3.16 M and HCl 2.2 N for 3 days. However, there were significantly different both for acid types and for the duration of hydrolysis. On hydrolysis for 5 days, the HCl treatment resulted higher yield compared to H2SO4. Meanwhile for arrowroot starch NP, there was no significant difference, even though there was a similar tendency, namely the treatment with H2SO4 and the longer the hydrolysis the lower the resulted yield. This was due to the longer hydrolysis time, the more components of starch were dissolved in the acidic medium so that the yield of the resulting nanoparticles becomes decreased. The effect of acid types was reported by Angelier et al. [12] that the production of nanocystal with H2SO4 was lower than HCl, but the final suspension was more stable using H2SO4 because of the presence of sulfate groups on the surface. The factors that influence acid hydrolysis are hydrolysis time, acid type, acid concentration, and incubation temperature [7].
Table 1. Effect of hydrolysis duration and acid type on the yield of taro starch NP

| Hydrolysis duration | Acid type | Mean |
|---------------------|-----------|------|
|                     | H₂SO₄     | HCl  |
| 3 days              | 40.592 a (A) | 34.443 a (A) | 37.518 A |
| 5 days              | 21.644 b (B) | 30.428 a (A) | 26.036 B |
| Mean                | 31.118 a  | 32.436 a  |

Remarks: the numbers followed by the same lowercase letter in the same row and the same capital letter in the same column were not significantly different based on the Duncan Multiple Range Test (DMRT) advanced test at the 5% level

Table 2. Effect of hydrolysis duration and acid type on the yield of arrowroot starch NP

| Hydrolysis duration | Acid type | Mean |
|---------------------|-----------|------|
|                     | H₂SO₄     | HCl  |
| 3 days              | 40.010 a (A) | 51.220 a (A) | 45.618 A |
| 5 days              | 40.129 a (A) | 38.056 a (A) | 39.093 A |
| Mean                | 40.072 a  | 44.639 a  |

Remarks: the numbers followed by the same lowercase letter in the same row and the same capital letter in the same column were not significantly different based on the Duncan Multiple Range Test (DMRT) advanced test at the 5% level

3.1. Morphology

The results of SEM analysis showed that natural taro starch granules (Figure 1) had an irregular polygonal shape. The size of micro taro starch granules in micro sizes ranged from 1.344 µm - 4.695 µm. This was consistent with the study of Jane et al. [13] reporting that taro starch had irregular, polygonal and small grain sizes in diameters ranging from 1 - 5 µm. While arrowroot starch granules (Figure 1b) had an oval shape. Native arrowroot starch had a granule size ranging from 12.34 µm - 28.46 µm. Similar results with arrowroot starch granule size varied between 5-50 µm [14]. The results showed that treatment with 3.16 M H₂SO₄ for 3 and 5 days (Figure 2) produced a much smaller and nanometer-sized particle size. The particle size of taro and arrowroot starch nanoparticles treated with different acid and hydrolysis duration shown at Table 3. This was due to severe erosion of the granule surface due to H₂SO₄ attack [15] and [16]. Whereas for the treatment of 2.2 N HCl for 3 days has not changed the morphology of arrowroot starch, but only experienced cracks, grooves (Figure 3). However, taro starch NP has been damaged but still agglomerated. This occurred because acid only attacking the amorphous region while crystalline regions remain [17] so that the granule shape did not change much [18].
Figure 1. Morphology of natural starch granule: (a) Taro, (b) Arrowroot (1000x)

Figure 2. Morphology of taro starch NP (a, c) and arrowroot starch NP (b, d) treatment of H2SO4 for 3 days (5000x) and 5 days (10000x)

Figure 3. Morphology of taro starch NP (a, c) and arrowroot starch NP (b, d) treatment of HCl for 3 days (1000x) and 5 days (5000x)

Table 3. Effect of hydrolysis on the particle size of native starch nanoparticle

| Sample          | Acid treatments | Hydrolysis duration (day) | Particle size  |
|-----------------|-----------------|---------------------------|----------------|
| Taro starch     | H2SO4 3,16 M    | 3                         | 395.1 nm       |
|                 |                 | 5                         | 358.0 nm       |
|                 | HCl 2,2 N       | 3                         | 586.1 nm       |
|                 |                 | 5                         | 695.5 nm       |
| Arrowroot starch| H2SO4 3,16 M    | 3                         | 648.4 nm       |
|                 |                 | 5                         | 382.2 nm       |
|                 | HCl 2,2 N       | 3                         | 10.48 µm       |
|                 |                 | 5                         | 938.1 nm       |
3.2. Size Distribution
The particle size distribution showed that taro starch NP treated with H2SO4 3.16 M have average particle size and PdI value of 379.2 nm and 0.480; meanwhile arrowroot have average size of 464.4 nm and PdI value of 0.621, respectively. The resulting particle size was still in nano size because it was not more than 1 micron. Modified starch ranged from less than 1000 nm involved as nanoparticles [7]. The Polydispersity Index (PdI) was a measure of the mass distribution of molecules in a particular sample. The results of the Polydispersity Index (PdI) showed that the dispersion value of starch nanoparticles was relatively high ranging from 0.480 to 0.621. The high PdI value showed that most starches were still agglomerated as shown from the SEM profile. The ideal PdI value for nanoparticles is below 0.3 that showed monodispersity [19].

3.3. Crystallinity
The results showed that beneng taro starch and natural arrowroot have a degree of crystallinity of 44.1% and 43.5%, respectively, which were indicated by a peak of 2θ, namely 15 ° C, 17 ° C, 18 ° C, 23 ° C. This showed that natural starch has crystalline type A. The process of acid hydrolysis can increase its crystallinity which is indicated by an increase in% crystallinity in H2SO4 taro starch to 87.3% and arrowroot HCL starch to 51.3%. The increase in crystallinity was caused by the presence of acid which attacks the amorphous starch group while the crystalline part remains so that it can increase its crystallinity [17].

![Figure 4](image)

**Figure 4.** Crystalline pattern of taro starch (natural) and taro starch NP treated with H2SO4

3.4. Thermal Properties
The results obtained showed that the peak temperature of the acidic hydrolysis of the starch was lower while the enthalpy tended to be higher. While the RVA results showed that the peak viscosity temperatures were respectively 96°C and 99°C. In line with the research of Aprianita et al [20] who reported that taro starch had a fairly high gelatinization temperature. This suggests that taro starch has higher stability during heating [21]. While natural starch has a lower enthalpy (ΔH) of 41.4946 J/g and 52.7016 J/g, respectively. This was because the energy needed to melt the acid hydrolysis starch was greater than the energy needed to melt natural starch. This occurred because acid hydrolysis can produce a higher proportion of crystalline than amorphous parts, therefore it can reduce peak temperatures and increase enthalpy. Table 4 presented the enthalpy value and peak temperature of taro and arrowroot starch natural and nanoparticles.
Table 4. Enthalpy and peak temperature of taro and arrowroot starch natural and nanoparticles

| Sample                           | Parameters     | ΔH (J/g) | Peak Temperature (°C) |
|----------------------------------|----------------|----------|-----------------------|
| Taro Beneng starch, natural      |                | 41.4946  | 92.30                 |
| Taro Beneng starch H₂SO₄        |                | 25.3068  | 81.50                 |
| Taro Beneng starch HCl          |                | 55.5006  | 87.99                 |
| Arrowroot starch, natural       |                | 52.7016  | 94.14                 |
| Arrowroot starch H₂SO₄         |                | 65.2330  | 88.10                 |
| Arrowroot starch HCl           |                | 94.4988  | 89.78                 |

4. Conclusion
The yield of arrowroot and taro starch nanoparticle (NP) was not significantly different for both 3 and 5 days using either using HCl or H₂SO₄ except for taro using H₂SO₄. Hydrolysis with HCl and H₂SO₄ produced starch NP with different characteristics. Treatment with HCl provides higher yield than that of H₂SO₄, but has a larger particle size. The average size of the taro and arrowroot starch nanoparticles produced by hydrolysis process with H₂SO₄ was 379.2 nm and 464.4 nm, with high polydispersity index of 0.480 and 0.621. Meanwhile, the crystallinity of taro and arrowroot starch NP increased from 44.1 and 43.5% become 87.3% and 51.3%, respectively. Starch nanoparticle by acid hydrolysis increased its solubility but decreased the swelling power and gelatinization temperature.

5. References

[1] Pareta R and Edirisinghe MJ. 2006. A novel method for the preparation of starch films and coatings. Carbohydr Polym 63:425–31.
[2] Tomask P and Schilling CH. 2004. Chemical modification of starch. Advances in Carbohydrate Chemistry and Biochemistry 59: 175–403.
[3] Xu Y, Ding W, Liu J, Kennedy JF, Gu Q and Shao S. 2010. Preparation and characterization of organic soluble acetylated starch nanocrystal. Carbohydr Polym 80:1078-84.
[4] Chen H, Weiss J and Shahidi F. 2006. Nanotechnology in nutraceuticals and functional foods. Food Technol 60(3):30–6.
[5] Lindeboom N, Chang PR and Tyler RT. 2004. Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. Starch. 56 (3-4): 89-99
[6] Raeker MO, Gaines CS, Finney PL and Donelson T. 1998. Granule size distribution and chemical composition of starches from 12 soft wheat cultivars. Cereal Chem. 75: 721–728.
[7] Le Corre D, Bras J and Defresne A. 2010. Starch Nanoparticles: A Review. Saint Martin d’Heres Cedex, France. Biomacromolecules, 11: 1139-1153.
[8] Szymonska J, Targosz KM and Krok F. 2009. Characterisation of starch nanoparticles. Journal of Physics 146.
[9] Angellier-Coussy H, Putaux JL, Molina-Boisseau S, Dufresne, Bertoft E and Perez S. 2009. The molecular structure of waxy maize starch nanocrystals, Carbohydrate Research, 344(12):1558–66.
[10] Le Corre D, Bras DJ and Dufresne A. 2012. Influence of native starch’s properties on starch nanocrystals thermal properties. Carbohydr Polym: 87: 658-666.
[11] Putaux JL, Molina-Boisseau S, Momaur and Dufresne A. 2003. Platelet nanocrystals resulting from the disruption of waxy maize starch granules by acid hydrolysis, Biomacromolecules, 4(5): 1198–202.
[12] Angellier H, Choisnard L, Molina-Boisseau S, Ozil P and Dufresne A. 2004. Optimization of the preparation of aqueous suspensions of waxy maize starch nanocrystals using a response surface methodology, Biomacromolecules. 5(4):1545–51.
[13] Jane J, Shen L, Chen J, Lim S, Kasemsuwan T and Nip WK. 1992. Physical and Chemical Studies of Taro Starches and Flours. Cereal Chemistry, 69(5): 528–35.
[14] Moorthy SN. 2002. Physicochemical and functional properties of tropical tuber starches: a review. Starch/Stärke, 54:559-94.
[15] Hoover, R. 2000. Acid-treated Starches. Food Review Int, 16: 369-392.
[16] Miao M, Jiang B, Zhang T, Jin Z, Mu W. 2011. Impact of mild acid hydrolysis on structure and digestion properties of waxy maize starch. Food Chem. 126:506-513.
[17] Srichuwong S, Isono N, Mishima T, Hisamatsu M. 2005. Structure of lintnerized starch is related to X-ray diffraction pattern and susceptibility to acid and enzyme hydrolysis of starch granules. Int J Biol Macromol37:115–21.
[18] Ferrini LMK, Rochaa TS, Demiate IM, Franco CLM. 2008. Effect of acid methanol treatment on the physicochemical and structural characteristics of cassava and maize starch. Starch/Starke. 60:417-25.
[19] Dahnier F, Margotteaux N, Ucakar B, Lecouturier N, Brewster M. 2009. Novel self-assembling PEG-p-(CL-co-TMC) polymeric micelles as safe and effective delivery system for Paclitaxel. European J Pharmaceutics and Biopharmaceutics. 72:230-8.
[20] Aprianita A, Purwandari U, Watson B and Vasiljevic T. 2009. Physico-chemical properties of flours and starches from selected commercial tubers available in Australia. International Food Research Journal 16: 507-20.
[21] Sasaki T and Matsuki J. 1998. Effect of Wheat Starch Structure on Swelling Power. Cereal Chemistry 75(4):525-29.

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