Synthesis of cassava starch based nano-hydrogels using gamma irradiation

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Abstract. Cassava tubers are one of the most starch producers in Indonesia, which is third after rice and corn. Cassava tubers starch can be used as raw material of natural polymer-based nanohydrogel synthesis. The use of natural polymers promises superior properties such as more eco-friendly, cheaper prices because their raw materials are available naturally in abundant quantities compared to synthetic polymers. To obtain similar properties to synthetic, this study modified starch using acid hydrolysis method for 2 hours and 24 hours. The ethanol precipitation method is then carried out to produce nano-particle starch. The process of nano-hydrogel formation was conducted by gamma irradiation. The results showed that the size of starch nanoparticles was between 14.97 - 492.7 nm. Nano-hydrogel with gamma irradiation treatment has 256.81% of swelling power, 83.35% of gel fraction, and 3.81 mJ of hardness. Meanwhile, the treatment without gamma irradiation has 365.47% of swelling, but has an unstable structure, this is indicated by 61.45% of gel fraction and 2.36 mJ of hardness.

1. Introduction

Natural polymers have been widely used as a raw material for the synthesis of superabsorbent polymers or well known as hydrogels. The use of natural polymers is advantageous in terms of economics because of its abundant availability, cheap, and renewable. In addition, the use of natural polymers is also more eco-friendly because of its degrade naturally [1]. Starch as one of the natural polymers produced from cassava tubers has a good prospect to be used as a raw material for nano-hydrogel synthesis. Based on FAO data (2017) cassava production reached 278 million tons, with the highest dry component being starch (starch), which was around 35% [2].

However, natural polymers such as starch have limitations in structure such as brittle, low resistance to shear stress, poor thermal properties, tend to be retrogradation, easily degraded, and dissolved in water. Therefore, in order to produce polymers that are able to absorb water with high capacity and not dissolve in it, modifications must to be done. The modification could be done by reduce the particle size of starch into nano size and increase cross linking between polymer chains. Nano-starch can produce hydrogel with higher water absorption ability and physical properties compared to micro starch [3]. Its cause of nano-starch has a ratio of surface area to a volume greater than micro-sized particles so that these particles have more pores, more reactive and easily form cross-links [4].

Crosslinking can be improved conventionally with the addition of chemical crosslinking agents and non-conventionally using gamma irradiation [5]. Both crosslinking methods have advantages and disadvantages, but non-conventional methods using gamma irradiation are seeded because of the process
takes place quickly and does not require chemicals as catalysts, crosslinking, and initiators which is generally toxic during the polymerization process. So that the resulting nano-hydrogel is safer to use. In addition, the crosslinking process with gamma irradiation leaves no residue on the process results, and the irradiation dose can be determined as needed [6].

Once polymer molecules are irradiated using gamma rays, interaction between polymer molecules and ionizing radiation will occur. As a result, the previously stable polymer chains are disturbed, thus forming radical ions. These radical ions then bind to each other to get their equilibrium state and polymers that have cross-linked three-dimensional structures (hydrogels) are produced [7]. Because this study uses nano-sized starch, the hydrogel produced from this study is said to be nanohydrogels. This research is important to improve the utility of starch as an eco-friendly hydrogel base material. The duration of acid hydrolysis and gamma irradiation was investigated in this study to determine its effect on water absorption ability and the physical properties of hydrogels produced.

2. Materials
The materials used in this study include cassava starch, aquadest, ethanol with concentration 96%, hydrochloric acid (HCl) solution with 2.2 N of concentration, NaOH with 5% of concentration. Instrument used were magnetic stirrer, digital balance, hot plate, cold room freezer and oven. Instrument used for hydrogel characteristics testing Particle Size Analyzer (PSA) Malvern, Texture Analyzer Texture Pro CT V1.2 Build 9, and Scanning Electron Microscopy (SEM) Zeiss EVO MA10 at Indonesian Center for Agricultural Postharvest Research and Development, Cimanggu-Bogor, West Java. The other was Irradiator ⁶⁰Co Gammacell 220 at Isotope and Radiation Application Center (PAIR) BATAN Lebak Bulus, South Jakarta.

3. Methods
Synthesis of starch based nano-hydrogels was carried out as follows:

3.1. Starch Hydrolysis
Starch hydrolysis was carried out using the method adapted from Faridah et al.[8]. Starch was dissolved in 2.2 N concentration of HCl, with a ratio of 1: 2 (w / v). The suspension is then soaked for 2 hours and 24 hours at a temperature of around 35 - 55° C. Furthermore, neutralization was carried out by adding 5% concentration of NaOH. The neutralized starch is then filtered and then dried in an oven at 55° C.

3.2. Production of Starch Nanoparticles
The ethanol precipitation method that adapted from Ma et al. [9] used to produce starch nanoparticles. Hydrolyzed starch was dissolved in distilled water at a ratio of 1:10 (w / v). Then it was heated while stirred using a magnetic stirrer at 85 ° C until it was gelatinized. Then the suspension was cooled at room temperature while 95% concentration of ethanol drops slowly. After the amount of ethanol added equal to the amount of starch solvent, the suspension was left to form a precipitate. The precipitant was then filtered and washed using ethanol to remove the remaining residual water was then washed used ethanol to remove water.

3.3. Nano-hydrogel Production Using the Gamma Irradiation Crosslinking Method
Production of starch based nano-hydrogels using a gamma ray irradiation method, modified from Erizal [10]. This process begins with dissolved nano-starch in distilled water with a ratio of 1/2 (w / v), then the solution was stirred at 55 ° C for 1 hour until homogeneous. After that, the nano-starch solution was put into plastic and sealed, then exposed to gamma irradiation at a dose of 20 kGy at a rate of 5 kGy / Hour. Then filtered and dried in an oven at 60 ° C. Then the swelling and gel fraction data were collected, texture analysis was carried out using a texture analyzer instrument.
3.4. Analysis of Swelling Power
Swelling power was measured by compared the mass of the sample after immersed in water (mₜ) to the initial mass of the sample (m₀). Determination of nano-hydrogel mass was carried out using the gravimetric method. Before the nano-hydrogel swelling mass was weighed, the water content of nano-hydrogel were removed by attaching filter paper to the nano-hydrogel surface. The swelling power was calculated by this equation:

\[ \text{Swelling (\%)} = \frac{mₜ}{m₀} \times 100\% \]

3.5. Analysis of Gel Fraction
The dried nano-hydrogel was weighed (m₀), and soaked in water for 24 hours then dried in an oven at 60° C for 6 hours [10]. Then the dry mass after immersion (mₙ) was weighed. Gel fraction could be determined by this equation

\[ \text{Fraksi Gel (\%)} = \frac{mₙ}{m₀} \times 100\% \]

4. Results and Discussion

4.1. Hydrolized Starch
Starch used in this study was prepared using acid hydrolysis for 2 hours and 24 hours. The hydrolysis process breaks the long chain amylose fraction and the branching point of amylopectin, that resulting starch with dominated by short chain amylose fractions. Our result demonstrated that there is no significant difference in the SEM image between 2 hours hydrolyzed starch and 24 hours hydrolyzed starch (Figure 1). This result indicated that, hydrochloric acid (HCl) during the hydrolysis process degrades the amorphous area of starch granules, so that the surface of the starch granules does not change shape of starch significantly as seen in Figure 1 [11]. Based on Figure 1, there are visible cavities in starch, especially in morphology of 24 hours hydrolyzed starch. Cavities are the origin of the breakdown of starch granules due to the acidification process [12].

![Figure 1. Morphology of hydrolyzed starch (a) 2 hours and (b) 24 hours with magnification of 4000 ×](image)

4.2. Starch Nanoparticle
Starch nanoparticles were produced using the ethanol precipitation method. The sized distribution of starch nanoparticles was measured by Malvern particle size analyzer (PSA) instrument. The results obtained by PSA in Table 1 and Figure 2 showed that the nano-starch from 2 hours hydrolyzed was in the range of sizes 14.97 nm to 492.7 nm with the most size in 33.04 nm. Meanwhile, the nano-starch from 24 hours hydrolyzed had particle size in the range of 35.53 nm to 450.2 nm with the most dominant size at 35.53 nm (Table 2 and Figure 3). Based on the both results obtained it can be concluded that the starch particles produced from the ethanol precipitation method have nano-size.
Table 1. Particle size distribution of nano-tapioca from 2 hours hydrolysis

| Peak | Size (d.nm) | % Vol | Σ | % Pd |
|------|-------------|-------|---|------|
| 1    | 14.97       | 1.5   | 14.8 | 14.8 |
| 2    | 33.04       | 59.9  | 7.103 | 21.5 |
| 3    | 492.7       | 17.3  | 120.9 | 24.5 |

Figure 2. Particle size distribution of nano-starch from 2 hours hydrolysis

Table 2. Particle size distribution of nano-starch from 24 hours hydrolysis

| Peak | Size (d.nm) | % Vol | Σ | % Pd |
|------|-------------|-------|---|------|
| 1    | 35.53       | 23.1  | 4.866 | 13.2 |
| 2    | 226.1       | 5.7   | 33.79 | 14.9 |
| 3    | 450.2       | 15.4  | 68.74 | 15.3 |

Figure 3. Particle size distribution of nano-starch from 24 hours hydrolysis
However, the data obtained had a fairly high polydispersity. This result indicates that the nano-starch produced in this study do not have a uniform size. This finding appropriate to SEM image in Figure 4. We speculate that this might be due to nano-starch produced from precipitation have not been completely separated and are still assembled to form agglomerations [13].

![Figure 4](image)

**Figure 4.** Morphology of starch nanoparticle with hydrolysis time of (a) 2 hours and (b) 24 hours with 4000 × of magnification

### 4.3. Analysis of Swelling Power

Swelling power was measured by calculating the percentage ratio of mass after immersion to the initial mass of the nano-hydrogel before being soaked in water. Figure 5 shows the kinetics swelling of nano-hydrogels from 2-hours hydrolyzed starch. Based on the results in Figure 5, gamma ray irradiation at a dose of 20 kGy causes a decreasing in swelling. This result is also shown in Figure 6 which shows the kinetic swelling of nano-hydrogel from 24 hours hydrolysis starch. The decrease in swelling caused by crosslinking between polymer chains due to the formation of radical ions due to gamma irradiation which inhibits the mobility of the polymer chain and reduces the penetration of water into the nano-hydrogel structure. In addition, the decrease in swelling value can also indicate that the irradiation dose used is too high, so that some of the polymer undergoes degradation [14]. This result consistent with the results of research conducted by Fekete et al. [15]. Giving gamma irradiation causes a degradation process that decreases the degree of polymerization and decreases swelling.

The comparison of the swelling results in Figure 5 and Figure 6 showed that nano-hydrogels produced from 2 hours hydrolyzed starch have higher swelling than nano-hydrogels from 24-hour hydrolyzed starch. The duration of starch hydrolysis affects the crystallinity of starch nanoparticles produced. The length of hydrolysis time determined the amount of the amorphous part of the starch lamella which was degraded. This is due in the acid hydrolysis process, the part which first attacked was an amorphous part, so at 24 hours hydrolysis, the part of amorphous lamellae has been degraded and leaves a part of crystalline lamella [13].
The amorphous part of starch granules tends to be composed of amylose, while amylopectin has a double the helix is generally in the crystalline area of starch. The degradation of amorphous parts of starch causes a reduction in the amount of amylose present in starch granules. As a result swelling decreases, because the part that plays a role in starch swelling is amylose [4]. However, the longer process of starch hydrolysis can increase the strength of nano-hydrogels.

4.4. Gel Fraction

Based on Table 3, it was found that gamma irradiation at a dose of 20 kGy was able to increase the 2 hours hydrolyzed starch nano-hydrogel gel fraction from 60.06% to 83.351% and from 59.65% to 78.906% for nano-hydrogel with 24 hours hydrolyzed. The gel fraction is the part that is not dissolved during the immersion process. This value shows the efficiency of the crosslinking process [16]. The results show that crosslinking using gamma irradiation increases the gel fraction, which means it can reduce the solubility of nano-hydrogel in water. Giving gamma irradiation can increase free radicals that trigger crosslinking, consequently forming a three-dimensional network that is insoluble in water [17]. The three-dimensional bond that formed causes the forces between molecules in the nano-hydrogel to be stronger, so that these nano-hydrogels are not easily dissolved in water and the mechanical strength is getting better [11].
Nano-hydrogel texture has been measured using a texture analyzer instrument. The results obtained are presented in Table 4. Based on Table 4, gamma irradiation method has been increased Hardness, cohesiveness, and springiness, and reduced adhesiveness of nano-hydrogels. According to Ghanbarzadeh et al. [18], crosslinking reactions in nano-hydrogels cause a more dense structure to form. So, the strength of nano-hydrogel will increase with gamma irradiation treatment. In Table 4, the nano-hydrogel hardness value can be significantly improved by gamma irradiation treatment. The result in Table 4 showed that hardness increased with the increasing of starch hydrolysis time. In the same crosslinking treatment, nano-hydrogels from 24 hours hydrolyzed starch had a higher hardness than nano-hydrogel from 2 hours hydrolyzed starch. Acid hydrolysis causes the degradation of amylose chains and branching of amylopectin found in the amorphous region and leaves crystalline regions, because the acid attacks the crystalline regions slowly in contrast to the amorphous regions which are more rapidly attacked [4]. With the degradation of the amorphous regions in starch granules, the starch particle size becomes smaller [19]. The small particle size causes the starch to react more easily and form crosslinking. This caused the hardness of the nano-hydrogel from 24 hours hydrolyzed starch higher than the hardness of nano-hydrogel from 2 hours hydrolyzed starch in the same crosslinking treatment. In addition, gamma irradiation increased the cohesiveness and reduce adhesiveness of nano-hydrogels.

Adhesiveness measure the interaction forces between unsimilar molecules [20]. Based on Table 4, it can be seen that the control treatments (2A and 24A) have very high adhesiveness values when compared to nano-hydrogels that have crosslinked. Adhesiveness show the solubility of nano-hydrogels in water. The high adhesiveness showed that nano-hydrogels are more easily degraded because they are easily soluble in water. Table 4 shows that cross-linking treatment using gamma irradiation agents can significantly reduce adhesiveness and increase cohesiveness. A decrease in the adhesiveness value has a good effect on the mechanical structure of nano-hydrogels. Meanwhile, cohesiveness as shown in Table 4 has an opposite relationship with adhesiveness. Cohesiveness is a measure that describes the strength of interactions between similar molecules. The high cohesiveness value shows better mechanical stability. Springiness showed the elasticity of a material when given a load. This value can be measured by measuring the recovery force possessed by the material after being given a compression force. Based on Table 4, nano-hydrogels which have the highest springiness value are nano-hydrogels produced from 2 hours hydrolyzed starch.
4.6. Morphological Analysis

Nano-hydrogel morphology was observed using the Scanning Electron Microscope (SEM) instrument. Morphological results show gamma irradiation samples have a finer morphology than control samples as shown in Figure 7. This is due to the crosslinking process of gamma irradiation causing polymer chains in the nanoparticle starch to interlock to form a three-dimensional structure [21]. The surface morphology results are in accordance with the results obtained from the swelling, gel fraction, and texture. Based on the formed image it can be seen that the nano-hydrogels produced from 2-hours hydrolysis process have more pores than nano-hydrogels from 24-hour hydrolyzed starch. This is indicated that the hydrogels produced from hydrolysis for 24 hours the amorphous portion of starch which plays a role in swelling and crosslinking have been degraded leaving a stable and difficult crystalline part to form bonds.

Table 4. Texture of nanohydrogels

| Sample Code | Hardness (mJ) | Adhesiveness (mJ) | Cohesiveness | Springiness (mm) |
|-------------|---------------|------------------|--------------|-----------------|
| 2C          | 2.36          | 2.00             | 0.05         | 0.07            |
| 2G          | 3.81          | 0.05             | 0.36         | 2.89            |
| 24C         | 4.03          | 1.70             | 0.03         | 0.16            |
| 24G         | 5.32          | 0.02             | 0.41         | 1.77            |

*2 : 2 hours hydrolyzed starch; 24: 24 hours hydrolyzed starch; C:Control; G:Gamma irradiation*
Based on the results of the study it can be concluded that, hydrolysis time affected the crystalinity of starch. The length of time for acid hydrolysis degraded the amorphous group of the starch, so that the swelling of nano-hydrogel decreased. Gamma irradiation decreases swelling of nano-hydrogel, increases the gel fraction, and improves the strength of the texture mechanically. Nano-hydrogel with gamma ray irradiation from 2 hours hydrolyzed starch is the most optimum treatment in this research.

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