The Effect of Cross-linking and Fatty Acid Addition on the Functional Properties and Digestibility of Tapioca Starch

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Abstract. The present work was carried out the effect of cross-linking and fatty acid addition on the functional properties and digestibility of tapioca starch. Tapioca starch was chemically modified using sodium trimetaphosphate (STMP)/sodium tripolyphosphate (STPP) and fatty acid addition used stearic acid (C18). Thermogravimetric analysis showed higher thermal stability for the modified granules compared to the native one. A-type crystalline was formed on native and cross-linking starch, then A-type and V-type crystalline polymorphs were formed between starch and fatty acids. The both modifications decreased solubility, swelling power, paste clarity, viscosity and the dual modification significantly decreased digestibility of tapioca starch.

1. Introduction
Starch is one of the sources of food that has high availability and can be used as a reserve of energy in the human body [1]. Starch was classified into three type of starch according to the timeline of digestible which are resistant starch (RS), slowly digestible starch (SDS), rapidly digestible starch (RDS) [2]. The cross-linking process is one of the chemical modifications that can strengthen bonds between starch molecules, both intra-bonding and inter-molecular. The effect of the crosslinked depends on the degree of modification, the source of the starch, and the cross-linking agent [3]. Cross-linking can inhibit the digestibility of α-amylase. Crosslinked starch is performed using sodium tripolifosfate (STTP) and sodium trimetapospat (STMP) [4].

In addition to use chemical modifications, starch can also be modified with the addition of fatty acids that can produce resistant starch through the formation of complex compounds between amylose and lipid [5]. Starch containing high amylose will make it easier to make complex, the optimum concentration for the ratio of amylose to fatty acids in forming complexes with starch is 10:1 (w/w) [6]. The formation of the complex amylose compounds with lipids can occur in lauric, myristic, palmitate and stearate acid [7].

In the present study, we prepare for tapioca starch with cross-linking and fatty acid addition to investigate the effects of the functional and digestibility properties. Determination the functional properties of starch modification results include swelling power, solubility, paste clarity and viscosity. The complex formation of amylose lipid compounds can be observed using X-Ray diffraction at an angle of 2θ around 22° and 24° [8], Thermal characteristics using Thermogravimetric Analyzer (TGA), morphology of its granulas using the Scanning Electron Microscope (SEM). This research is expected to provide information about the physicochemical characteristics of these modifications and can be a basis in developing functional food as well as increasing the value of tapioca starch in food industry.
2. Materials and Methods

2.1. Materials
Tapioca starch, sodium trimetaphosphate (STMP), α-amylase heat stable, sodium tripolyphosphate (STPP), distilled water, ethanol, sodium sulfate, aluminium oxide, hydrochloric acid, 3,5-dinitrosalicylic acid (DNS), hexane, sodium hydroxide, potassium sodium tartrate and stearic acid.

2.2. Methods

2.2.1. Preparation of cross-linked tapioca starch. 15 g STPP (5%) and 6 g STMP (2%) dissolved in the distilled water 300 mL containing 15 g of sodium sulfate (5%). Tapioca Starch (50 gr) added into the solution and added the distilled water to 667 g. adjust pH to 11 with NaOH 1 M. Porridge in the suspense is stirring for 1 hour in 45 °C, strain the slurry and dried in the oven at 40 °C for 5 hours, then heated 2 hours at 130 °C. Chill the slurry at room temperature, mix with 350 mL distilled water, adjust pH to 6.5 with HCL, centrifuge the sample (1,500 x g, 10 min). Wash the deposition with 600 mL distilled water 3 times and dried at 40 °C.

2.2.2. Preparation of fatty acid addition tapioca starch. 3% Stearic acid was added in hexane as much as 150 mL, then add tapioca starch as much as 100 g. The mixture is stirring at room temperature for 12 hours at 100 rpm until the hexane evaporates naturally, then the remaining hexane is removed using the oven in 70 °C.

2.2.3. Preparation of dual modification. Dual modifications like cross-linked fatty acid addition and fatty acid addition cross-linked starches were prepared using the methods for cross-linking and fatty acid addition alternatively. For cross-linking, STMP and STPP were added in the same quantity and ratio as explained previously in “Crosslinking” section, stearic acid was added in the same quantity as explained in “Fatty acid addition” section.

2.2.4. Paste clarity. 0.25 g starch sample added to 25 mL of distilled water. Then, heat in boiling water for 30 minutes with a little stirring. After that, cool at room temperature for 10 minutes. Pasta clarity was measured using UV-Vis instruments at wavelengths of 650 nm.

2.2.5. Solubility and swelling power. Starch 0.5 g was mixed with distilled water 15 mL and kept in the water bath at a temperature of 85°C for 30 minutes. Chilled the suspension at a room temperature (25°C) and centrifuged at 1,000 rpm for 15 minutes to separate sediment and supernatant. The solubility of starch was counted from supernatant which was dried at a temperature of 110°C, for one night. The weight of starch solubility on supernatant was measured with the equation below.

\[
\text{Solubility} = \frac{\text{weight of dry paste starch}}{\text{weight of dry starch}} \times 100 \quad (1)
\]

\[
\text{Swelling} = \frac{\text{weight of wet paste starch}}{\text{weight of dry starch} \times (100 - \% \text{sol})} \times 100 \quad (2)
\]

2.2.6. Viscosity. 3 gr starch was added to 300 mL distilled water in a beaker glass. Then, it is heated for 10 minutes. The spindle number 1 is mounted on viscometers and set to30 rpm speed. Spindle is lowered in the sample. The viscometer is switched on and readings are performed when the number of indicator needle is stable.
2.2.7. Digestibility. The enzyme was α-amylase heat stable (activity of 37 KNU). The sample 0.1 g was weighed and added with 10 mL of distilled water in the tube. It was heated for 30 minutes at a temperature of 90°C, and added with 5 mL of α-amylase and adjust to pH 4 and incubated at a temperature of 90°C for 30 minutes. Each sample was made twice in which one of them was blank. After that, 1 mL of the incubation sample was put into the closed reaction tube which contained 2 mL of DNS (dinitrosalicylic acid). Sample solution was heated at 100°C for 10 minutes and cooled with the running water. Then, added 10 mL of distilled water and made homogeneous with vortex mixer. The formed colours were red and orange, in which the absorbance was measured at wavelength of 520nm using spectrophotometer of UV-Vis. Digestibility was measured with the equation below.

\[
\% \text{ Digestibility} = \frac{\text{Abs modified starch}}{\text{Abs pure starch}} \times 100
\]

(3)

2.2.8. X-ray diffraction (XRD) measurement. Characterization with XRD was conducted to identify starch crystallinity. The sample was pasted to the glass, mounted to the sample holder with the aid of adhesive wax, and put on the sample stand. The measurement was conducted at the value of 2θ from 10° to 90°. Tool was operated using the radiation of Cu Kα (λ = 0,15406 nm) at 40kV and 80mA.

2.2.9. Scanning electron microscope (SEM). The granular structure was observed using a scanning electron microscope (SEM). The samples were then evenly distributed on SEM specimen stubs with double adhesive tape and coated with a 10 nm gold layer. Representative micrographs were taken for each sample at magnifications of 1000× and 2000×.

2.2.10. Thermogravimetric analysis (TGA). The measurement using TGA was conducted to see thermal stability between native starch and the modified starch. The sample was put into thermocouple made of aluminium. Thermocouple which contained sample and reference material was put into the instrument. The analysis was conducted at a temperature of 30-550°C with the adjustment of temperature increase of 10°C/minute. The percent weight loss was calculated by software and the range calculation of percent weight between 230°C and 380°C.

3. Results and Discussion

3.1. Paste clarity
Determination the clarity of paste done by making 1% flour solution (w/w) then heated and stirring for 30 minutes. Then, the clarity is determined by the transmittance that produced using the Uv-Vis spectrophotometer at a wavelength of 650 nm using distilled water as a blank.

![Figure 1. The result of clarity of paste](image-url)
The clarity of paste relates to the ability of paste to experience gelatinization. When the flour has gelatinization, the granule will expand (swelling) and making the light can releasing it rather than reflecting the subsequent flour will dissociate and the ability to reflect light disappears. So, flour that is not good in swelling ability have lower clarity. The same results of a decline in the clarity of starch paste due to a crosslinking reaction was also obtained on potato starch and corn starch [9,10]. The structural changes in starch granules by cross-linking make some starch remain intact by heating a certain temperature resulting in a decrease in the clarity of pasta [9] although not significantly lowered. While the formation of complex compounds lowers clarity because it strengthens the intermolecular bond due to the formation of amylose-lipid compounds, so the granules are not easy to swell and the viscosity decreases consequently, then the clarity also decreases significantly.

3.2. Swelling Power and Solubility
Swelling power is influenced by amylopectin. Amylopectin resides in an amorphous area. The more amylopectin that is in starch, then the amorphous area will be wider so that water absorption will be greater and swelling power increases. Based on result in Figure 2, that pure starch has the highest value of swelling power, likely because it has a comparison of greater amylopectin levels than the modified starch. While the modified starch decreases the value of swelling power.

![Figure 2. The Result of Swelling Power](image)

Swelling power of the crosslinked starch decreased due to the presence of phosphate molecules tied to the hydroxyl group of starch molecules so that the starch granule is resistant to swelling and requires higher temperature for hydration [11]. While in starch with the formation of complex amylose-lipid compounds, the formation of complexes in the flour decreased swelling power. This is because amylose and lipid interact due to the pull of the opposite charge and form an inclusion complexes and this limits the swelling [12].

Solubility is the ability of a material to dissolve in the water. Cross-linking reactions can increase the bonding between starch molecules and maintain the integrity of starch granules due to the addition of covalent bonds through the phosphate group [13]. The formation of cross-linking inter-chain starch causes the disintegrating of starch granules limited during heating so that the value of starch solubility decreases compared with pure starch. While in starch with the formation of complex compounds, especially with the presence of lipids that can be complexes that are not soluble in water. This is evidenced by the decrease in the value of solubility which is very significant when compared with pure starch.
3.3. Viscosity
Modified starch has decreased viscosity compared to pure starch. In Figure 4, pure starch has the highest viscosity of 33.6 mPa.S, while the modified starch has a viscosity of between 32.8-13.4 mPa.S. The modified cross-linking starch can maintain viscosity so that there is no difference with pure tapioca starch. Starch with the formation of amylose-lipid complex compounds as well as both dual modifications have lower viscosity. Decreased viscosity due to the formation of complex amylose-lipid compounds resulted in a weakened starch granule structure and inhibits the swelling of starch.

3.4. Digestibility
The digestibility power is performed In Vitro using α-amylase enzymes. The enzyme α-amylase is tasked to bypass the alpha bonding chain of polysaccharides in starch into shorter chains such as glucose and maltose. The detection of digestibility by enzymes is used in photometric way using dinitrosalicylic compounds. This method was chosen because it was easy to do, the results were quickly obtained and cheap. Dinitrosalicylic acid can react with free glucose and produce gluconate acid. The reaction scheme that occurs can be seen in Figure 5.
The results of the digestibility calculation are presented in Figure 6. The results from the table obtained there is a decrease in the digestibility of modified starch compared with without modification. The highest gastrointestinal decline occurred in the dual modification 1, that is a crosslinking modification and continued with fatty acid addition. This result from the change in the physicochemical properties experienced by starch. Increased thermal endurance and decreased swelling ability makes starch more resistant to the gelatinization process. Swelling of starch granules can facilitate access from digestive enzymes to the granule interior so as to increase the resulting sugar levels [14]. Because the process of gelatinization does not go well, it does not form strands of chain of polysaccharides that can be accessed enzymes to produce sugar. Thus, it produces a significant decline.

Figure 6. The result of digestibility

3.5. X-ray diffraction (XRD) measurement

Based on figure 7, in pure starch and crosslinked starch, there is a strong peak on the angular diffraction 2θ in the area 15° and 23° which corresponds to the double peaks in the area 17° and 18°. This indicates that there is A type of starch structure. The same pattern is also reported that pure cassava starch shows crystalline structure of type A starch. The pattern of pure and modified XRD starch does not indicate a significant difference except the peak sharpness at 2θ = 15° and 23° [15]. The cross-linking modification only replaces the starch functional group in the Amylopectin and amylose chains, thereby strengthening the starch granule without forming a new crystalline area [16].

Starch with modifications to the formation of a complex amylose-lipid compound, a single fatty acid indicates a sharp peak at 21.6° with a strong diffraction signal, and four other peaks around the area 15°, 22°, 23° and 24°. In the area 22° and 24° indicates the presence of crystalline pattern type V that indicates the existence of complex amylose-lipid compounds [8]. While at peak sharpness 15° and 23° indicates crystalline type A. Similarly, dual modifications 1 and dual modifications 2 also have a strong signal in the 21.6° area that shows single fatty acids, as well as there are peaks in areas 22° and 24° and there is a peak sharpness of 15° and 23°, indicating crystalline pattern of type A and V.

Figure 7. The result of XRD
3.6. Scanning Electron Microscope (SEM)
Microscopic testing of starch granules of tapioca grains shows round shaped granules, smooth, granule size diameter of 4-35 μm and usually there are some truncated rounded parts [17]. Based on the image below, the morphological form of starch of tapioca changes with modification. Cross-linking reactions cause the surface of starch granules to be coarse and the presence of black holes on the surface of starch granules. The black zone seemed to exhibit little fragmentation and formed a deep groove on the starch granules. The same results were also reported that the banana starch and corn tied up with a mixture of sodium trimetaphosphate and sodium tripolyphosphate indicate black zones on the surface of the starch granule [18,19]. Whereas in modification of the formation of complex amylose-lipid compounds also produce irregular and perforated forms. In double modifications also produce granules that have black, perforated and irregular zones. This indicates that with the presence of starch modification, the amount of whole starch grains decreases.

![Figure 8](image)

**Figure 8.** The result of SEM (1 (pure), 2 (CL), 3 (Dual 1), 4 (FAA) and 5 (dual 2))

3.7. Thermogravimetric analysis (TGA)
Weight loss of pure starch according to [4] is 98.31%. The TGA test results shown in the image below, the result in a percent weight loss starch modification of the cross-linking decreased to 95.08% while the modification of amylose-lipid starch became 95.74%.

![Figure 9](image)

**Figure 9.** The result of TGA (Crosslinking)
The presence of cross-linking reactions and fatty acid addition was able to strengthen starch granules thereby improving the thermal stability of pure tapioca starch for the better.

4. Conclusion
This study made it clear that modified starch decreases swelling power, solubility, paste clarity and viscosity and digestibility when compared to the pure starch. Modified starch produces crystalline type A and type V. The result of thermogravimetric analysis, modified starch has better thermal resistance.

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