Effect of different amount of cross-linker and catalyst on modified cassava towards its chemical characteristic

D Sondari\textsuperscript{1,*}, P Amanda\textsuperscript{1}, R Suryaningrum\textsuperscript{1}, D Burhani\textsuperscript{1}, D A Pramasari\textsuperscript{1}, A A Septevani\textsuperscript{2}, W K Restu\textsuperscript{2}, E Agustian\textsuperscript{2}, Y Irawan\textsuperscript{2} and M Oktaviani\textsuperscript{1}

\textsuperscript{1}Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Kawasan Cibinong Science Center, Jl. Raya Bogor Km.46, Cibinong, Bogor, West Java, 16911, Indonesia
\textsuperscript{2}Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Kawasan Puspiptek Serpong, South Tangerang, Banten, 15314, Indonesia
\textsuperscript{*}E-mail : sondaridewi@gmail.com

Abstract. The experimental works involved preparing modified cassava using cross-linker sodium trimethyl phosphate (STMP) and catalyst (sodium chloride) towards its chemical characteristic. In this study, the number of cross-linker and catalyst were varied to determine the effect of each amount of cross-linker and catalyst towards the value of solubility, swelling power, freeze-thaw, and FTIR analysis. Their structural and functional properties were also characterized by Fourier Transform Infrared (FTIR). This study found that the properties of modified cassava containing 2\% (w/w) of the catalyst were less effective than the modified cassava comprised of 4\% (w/w) sodium chloride. The 2\% (w/w) content of cross-linker in the modified cassava model was the best amount of STMP to be used in this process.

1. Introduction
Starch is a digestible polysaccharide and the second-largest carbohydrate component after cellulose. It is a homopolymer of glucose or homopolysaccharide with α-glycosidic bonds. In addition, starch is abundant, renewable, and biodegradable, found in roots, stems, and seeds from several plants [1-4]. The tapioca starch component ranges from 80-89\% with amylose levels of 20-27\% [5]. The difference between amylose and amyllopectin levels in tapioca starch will affect its ability as a drug-carrying matrix [6]. According to previous researcher, amylose has a linear structure to encapsulate drugs, so that starch with a high amount of amylose can function as a useful drug delivery matrix [7]. According to Sujka et al., the factors that influence starch effectiveness of starch for a drug delivery matrix are the amount of amylose and the starch granules size [8]. The large size of the starch granules can indicate the large porosity as a place for the reaction between starch and drugs. One way to increase the shaft size of starch is to react optimally to form complexes with drugs in encapsulation by chemically modifying the cross-linking method [8].

Cross-linking is a chemical modification technique for starch strengthening chemical bonds in granules to form an intramolecular bridge. The factors that influence the cross-linking reaction are the number of cross-linkers, pH, temperature, and reaction time [9-11]. This method is widely used because it is easy and cheap, but the process is a slow reaction, so it is necessary to add some catalysts. The types of cross-linkers commonly used are bifunctional and polyfunctional compounds. The types
of food-grade cross-linkers used in starch modification for food and pharmaceutical products are sodium trimetaphosphate, phosphorus oxychloride, and sodium tripolyphosphate [12].

This research aims to modify tapioca starch, which will be used as a drug delivery matrix using the cross-linker method. The cross-linker used in this study was sodium trimetaphosphate (STMP), while sodium chloride was the catalyst.

2. Material and Methods

2.1. Material. Cassava starch (Cap Dua Tani) was purchased from a local market in Bogor. Sodium trimetaphosphate (STMP), NaOH, NaCl, and HCl are from Merck and used without purification. Distilled water was used for the whole process.

2.2. Methods. Cross-linked cassava starch (CLCS) with STMP was prepared using the previous methodology by previous research with some modification [12]. Formulations of modified starch consist of native cassava starch (NCS), with different amount of STMP (0.5, 1.0, and 2.0 g) and NaCl (2.0 and 4.0 g) were mixed in 20 mL of distilled water and stirred at 300 rpm, heated in 70°C water bath for 1 hour. After that, the pH was adjusted to 11.0 with adding 0.1 N NaOH. The slurry was kept stirred for 5 hours. Then the obtained suspension was neutralized to pH 6.5 with adding 0.1 N HCl and centrifuged. And then the starch dried at 50°C for 24 hours.

2.3. Characterization

2.3.1. Swelling power and Water Soluble. Starch (0.1 g) was weighed into a centrifuge tube, and 10 mL distilled water was added. The samples were mixed using a vortex mixer for 10 seconds and incubated at an 85°C water bath for 30 minutes mixed with several times (5, 15, 25 minutes). The tubes were cooled to room temperature using an ice-water bath and centrifuged at 2000 rpm for 30 minutes. The supernatant was removed, and the remaining sediment in the tube was weighed (Ws). The supernatant was dried to constant weight (W1) in a drying oven at 100°C [13]. The swelling power (SP) and water-soluble index (WSI) were calculated using the following equation:

\[ SP = \frac{Ws}{[0.1 \times (100\% - WSI)]} \text{(g/g)} \]  
\[ WSI = \frac{W_s}{W_1} \times 100\% \]

2.3.2. Freeze thaw stability. An aqueous suspension of CLCS was heated at 95°C under constant mild agitation for 30 min and then cooled in an ice-water bath. The paste was weighed in centrifuge tubes and freezing at -18°C for 24 hours. The sample should be thawing at 30°C for 1.5 hours, followed by centrifugation at 3000 rpm for 10 minutes. The supernatant eliminated from the gel was weighed, then expressed as % syneresis [14].

\[ \% \text{ syneresis} = \frac{\text{supernatant weights after freeze – thaw (gram)}}{\text{gel weight (gram)}} \times 100\% \]

2.3.3. Fourier Transform Infrared Spectroscopy. Functional group analysis on native sago and cross-link cassava starch was carried out using FTIR (Spectrum Two, Perkin Elmer) and recorded over a range of wavenumbers from 400 to 4000 cm⁻¹.
3. Results and Discussion

Based on Table 1, it can be seen the swelling power and water solubility of native cassava and modified cross-link cassava starch. Modified cross-link cassava starch (CLCS) with variation concentration of catalyst and cross-linker has a more excellent swelling power value than that native cassava starch (NCS) but not significantly. Modified cassava starch has a swelling power value between 8-9%, depending on the number of cross-linkers and catalyst used. Solubility is the ability of starch to dissolve in water. Native starch is slightly soluble in water, so that its use is limited, while phosphate starch is expected to have a higher solubility than native starch so that its utilization in the food and pharmaceutical industries will be more effective [6].

| Sample          | Swelling power (%) | Water soluble index (%) |
|-----------------|--------------------|-------------------------|
| NCS             | 7.99               | 15.65                   |
| CLCS 4:0.5:2    | 8.09               | 19.85                   |
| CLCS 4:0.5:4    | 8.61               | 19.52                   |
| CLCS 4:1:2      | 8.62               | 21.33                   |
| CLCS 4:1:4      | 8.52               | 20.77                   |
| CLCS 4:2:2      | 9.08               | 20.50                   |
| CLCS 4:2:4      | 19.10              | 26.37                   |

Modified tapioca starch has a higher swelling power value compared to native cassava starch. Swelling power or water holding capacity is the starch's ability to hold water, causing the starch granules to expand. Phosphate starch has a more excellent swelling power value than native starch because phosphate starch has more hydrogen bonds and covalent bonds to easily bind with water [9]. Swelling power is influenced by several factors, such as protein-lipid complex and amylose-amylopectin ratios. Swelling power is an essential property that starch can function as a matrix in drug delivery systems. When the gastrointestinal fluid penetrates the drug powder in the body, it enters the matrix and causes its structure to expand. This will push the drug out of the matrix and reach the target organs. Then the starch structure will erode itself due to rubbing against the liquid. This mechanism is called the initial burst release and self-erosion matrix [15].

Based on Table 1, it can be concluded that phosphate starch resulting from modification has a higher solubility value than that native starch. According to Guo et al. [16], starch phosphate has a strong affinity for water molecules because the covalent bonds formed between starch molecules and STMP molecules are polar covalent bonds that have electronegative differences. This causes strong molecular interactions with polar solvents such as water. The following factors that influence starch solubility are molecular weight, starch load, and starch structure [16].

Freeze-thaw stability is a parameter that states the stability of starch when frozen and thawed. Starch with high functional values must have stability under various extreme temperature conditions. When the starch goes through the shipping and handling process, the quality of the starch does not decrease. Freeze-thaw stability analysis was expressed in percentage (%) syneresis. The smaller the percentage syneresis value, the greater the freeze-thaw stability value [17]. The percentage of syneresis results from freeze-thaw stability analysis can be seen in Table 2.

Syneresis shows the amount of water separated from the starch paste when subjected to freezing and thawing treatments at extreme temperatures for a short period. A considerable syneresis value will increase the tendency of starch to retrograde. Retrogradation is the reunification of starch amylose molecules in order to form a gel. This occurs after the starch binds to water. Retrogradation is a sign of damage to the starch structure [17].
Cross-linking is one way to reduce the percentage syneresis of starch so that it does not undergo retrogradation. The cross-linker will block the starch chain with the newly inserted functional group, preventing the amylose chain's re-association. Starch as a drug-carrying matrix is expected to have a small percentage of syneresis so that starch has resistance to extreme temperature treatment [17].

Table 2. Syneresis analysis of NCS and CLCS

| Sample          | Syneresis (%) |
|-----------------|---------------|
| NCS             | 59.91         |
| CLCS 4:0.5:2    | 55.71         |
| CLCS 4:0.5:4    | 41.48         |
| CLCS 4:1:2      | 47.28         |
| CLCS 4:1:4      | 40.90         |
| CLCS 4:2:2      | 35.23         |
| CLCS 4:2:4      | 17.29         |

Functional group analysis using FTIR to determine the success of the modification process with P=O, and P-O-C groups, which indicate the cross-linking of starch with the phosphate groups from STMP. The mechanism in the modification of the starch cross-linking technique is cross-linking between starch and its intermolecular and intramolecular cross-linkers. Starch has a hydroxyl group on carbon numbers 2, 3, and 6. On that carbon, when the cross-linking process occurs, there will be a release of hydrogen atoms in the hydroxyl groups of carbon numbers 2, 3, and 6, which are then replaced by STMP functional groups in the form of P=O and P-O-C [18]. The FT-IR spectra of the NCS and CLCS samples are presented in Figure 1 and 2.

![Figure 1. Spectra FTIR of native cassava starch (NCS) and cross link sago starch (CLCS 4:0.5:2; CLCS 4:1:2 and 4:2:2)]](image)

The determination of functional groups in the FTIR graph above refers to the previous researcher [12, 19, 20]. The functional groups of tapioca starch have similar intensities for both native starch and modified starch. Starch has several OH-groups, which showed in wave numbers 3500-3200 cm\(^{-1}\), which has a medium intensity and broad in shape. The –OH functional group comes from the alcohol bound to carbon numbers two, three, and six. Another functional group is the –CH alkane, which reads the wave number 2980-2800 cm\(^{-1}\) with weak intensity. The functional group –C-C- on starch reads
wave number 1700-1580 cm\(^{-1}\) with a weak intensity indicating the bonds in each carbon in starch. The functional group –C-O reads the wave number 1380-1290 cm\(^{-1}\) with weak intensity. The C-O-C functional group is an ether functional group present in starch to indicate an acetal group's presence, read in wave numbers 1150-960 cm\(^{-1}\) with medium intensity.

![Figure 2](image)

**Figure 2.** Spectra FTIR of native cassava starch (NCS) and cross linked sago starch (CLCS 4:0.5:4; CLCS 4:1:4 and 4:2:4)

The functional group P=O, located at wave number 1285-1255 cm\(^{-1}\), shows the bond in the phosphate group's bound, which is substituted with the hydroxyl group on the starch carbon, weak to medium intensity. Tapioca phosphate starch has a medium intensity; meanwhile, starch phosphate from sago has a weak intensity. P-O-C functional group is a functional group that connected the phosphate group with starch carbon numbers 2 and 6, located at a wavelength of 1200-1090 with high intensity (Table 3).

**Table 3.** FTIR characteristic of NCS and CLSS

| Samples (cm\(^{-1}\)) | NCS 4:0.5:2 | CLCS 4:0.5:4 | CLCS 4:1:2 | CLCS 4:1:4 | CLCS 4:2:2 | CLCS 4:2:4 | Characteristics   |
|------------------------|-------------|-------------|-------------|-------------|-------------|-------------|------------------|
| 3270                   | 3263        | 3264        | 3272        | 3269        | 3270        | 3268        | O-H groups       |
| 1336                   | 1335        | 1335        | 1306        | 1335        | 1303        | 1302        | C-H bending      |
| 1149                   | 1149        | 1149        | 1150        | 1150        | 1150        | 1149        | C-O stretching   |
| 1635                   | 1635        | 1635        | 1635        | 1635        | 1635        | 1635        | C-C             |
| 2930                   | 2929        | 2929        | 2931        | 2932        | 2930        | 2928        | C-H stretching   |
| -                      | -           | -           | 1266        | 1267        | 1267        | 1267        | P=O             |
| -                      | -           | -           | 1058        | 1066        | 1058        | 1058        | P-O-C           |

4. Conclusion
Modification of the casava has been successfully carried out by sodium hydroxide as a catalyst and STMP as a cross-linking agent. The concentration of the catalyst and the cross-linked agent affect the chemical properties of the modified starch. The 4% catalyst concentration has better chemical properties than the 2% catalyst concentration and the cross-linked agent’s concentration at 2%. A new peak can be seen at new bands related to phosphorylation. The intensity of the vibration peak of P=O
bending at 1285–1255 cm\(^{-1}\) and P-O-C stretching at 1200-1090 cm\(^{-1}\) appeared in the spectra of the samples. The spectra from Fourier transform infrared (FTIR) spectroscopy confirmed that the native cassava starch was successfully modified.

5. References
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