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Research Paper

Improving the Characteristics of Edible Film Using Modified Cassava Starch Over Ethanol Precipitation

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Abstract

Cassava starch has been widely explored as film forming material, however its hydrophilicity restricts its application. Hydrophobic material such as modified starch can be used in the film elaboration to improve its quality. This study aims to examine the effect of modified cassava starch concentration on the physical, barrier, and mechanical properties of edible films. A completely randomized design was used with 5 concentrations of modified starch at 0%, 5%, 10%, 15%, and 20% and 4 repetitions to obtain a total of 20 experimental combinations. The ANOVA showed that the modified starch concentration affected the compressive strength, thickness, transparency, and solubility of the edible film, but has no effect on its water vapor transmission rate (WVTR). Usage of 20% modified starch gave the product with the best characteristics at 0.22 mm thickness, 89.45 N/m^2 strength, 6.484%/mm transparency, 52.19% solubility, and 21.00 g/m^2.hour WVTR.

Keywords

Cassava Starch, Precipitation, Modified Starch, Edible Film

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1. INTRODUCTION

Cassava (*Manihot utilissima*) is one of the food crops with the highest production in Indonesia, and its tubers contain starch as the main component. It also has a high amylose content, which allows it to be used as raw material for edible films. The films can be used for packaging to resist mass transfer or as a carrier for food additives. The use of cassava starch as a single main ingredient produces films with low barrier and mechanical properties. To improve this weakness, researchers suggest a combination of several components to produce edible films (Zhou et al., 2021; Pérez-Vergara et al., 2020; Silva et al., 2019; Mantovan et al., 2018; Ulyarti et al., 2022). Some studies stated that the addition of modified starch with a different morphological shape or size can overcome these problems (Ulyarti et al., 2022; Wolf et al., 2018; Roy et al., 2020; Farrag et al., 2018; Ulyarti et al., 2020).

Modified starch produced from an acid hydrolysis of starch is shown to decrease water vapor transmission rate and increase film strength when incorporated to a film (Roy et al., 2020). This might be due to both a more hydrophobic nature of the modified starch and the fact that the incorporation of the modified starch with nano sized particles into film produces a more compact film (Le Corre and Angellier-Coussy, 2014). Starch modification using alcohol precipitation, on the other hand, produces similar properties of modified starch as acid hydrolysis modification but gives higher yields. Furthermore, alcoholic precipitation is more simple and cheaper than acid hydrolysis method. These are the reason for using modified starch from alcoholic precipitation for composite film. Precipitation method in starch modification involves the combination of physical and mechanical treatment of starch. The substrate is gelatinized in the presence or absence of surfactant and continues to undergo rapid stirring during the addition of non-solvent reagents, which causes retrogradation (Qin et al., 2016; Saari et al., 2017). The ratio of a non-solvent ethanol reagent to starch solution influences the morphology and the size of modified starch using the precipitation method (Chin et al., 2011).

A composite film produced from native cassava starch and the modified form through the alcoholic precipitation method using a hot plate at 100°C showed a better water vapor transmission rate (WVTR) and strength (Ulyarti et al., 2020). Similar results have been reported for composite film from corn and pea starch (Farrag et al., 2018). Therefore, this study aims to examine the effect of modified cassava starch concentration on the physical, barrier, and mechanical properties of edible film, as well as to determine the modified starch concentration that produces film with the best characteristics.

2. EXPERIMENTAL SECTION

2.1 Materials

This study used cassava tuber aged 5-6 months, which was harvested at Simpang Rimbo Jambi. The chemicals glycerol, absolute ethanol, calcium chloride, sodium chloride, and $Mg(NO_3)_2$ were from Merck.

2.2 Cassava Starch Extraction

The cassava tubers were peeled, washed, and grated. They were then mixed with water in a ratio of 1:2, and mashed using a blender. The cassava pulp was filtered using a 200-mesh sieve, and the filtering results were deposited for 4 hours. The supernatant was then discarded, while the precipitate formed at the bottom of the settling basin was washed with water and redeposited for 30 minutes. Subsequently, the starch precipitate was separated from the supernatant, dried using a drying oven at 50°C for 18 hours, and sieved using a 60-mesh sieve.

2.3 Preparation of Modified Cassava Starch

The preparation of modified starch followed the method described by Chin et al. (2011). A total of 1 gram of starch was dissolved in 100 mL distilled water, and then heated at 100°C using a hot plate for 30 minutes with constant stirring. Furthermore, the starch solution was added dropwise to a 500 mL of 96% ethanol with continuous stirring. The solution was left for 8 hours at room temperature with constant stirring and then centrifuged at 2,500 rpm for 15 minutes. The washing of the precipitate was carried out with absolute ethanol 3 times, followed by drying using cool-dry air in a refrigerator. The modified starch was then sieved with a 60 mesh sieve.

2.4 Preparation of Modified Cassava Starch Composite Film

The preparation of composite film followed the method described by Ulyarti et al. (2020). The composite films used modified starch at different concentrations, i.e., 0%, 5%, 10%, 15%, and 20% of the total starch. The composite films were prepared using a mixture of starch and modified starch (4 g), water (143 g), and glycerol (3 g).

The native cassava starch was stirred in distilled water using a magnetic stirrer to form a suspension. It was then heated using a hot plate at 80°C with continuous stirring for 40 minutes, followed by the addition of modified starch. The solution was then homogenized using a vortex without heating. A total of 25 gram film solution was poured into a petri dish with a 9.2 cm diameter and then dried in an oven at 60°C for 24 hours. The edible films were placed in a desiccator for 3 days, removed from the mold, and stored in a desiccator containing a saturated solution of $Mg(NO_3)_2$ for 2 days before analysis.

2.5 Starch Granule Morphology and Edible Film

Starch and edible film were imaged using a Scanning Electron Microscope (SEM) (model JEOL JSM 6510 LA). Before the analysis, the starch samples were dispersed with alcohol and coated with gold powder. Images were then taken at 500x and 1000x magnification on the surface of the edible film as well as its cross-section.

2.6 Particle Size Distribution

The size distribution of starch granules was determined using SEM images of the starches and analyzed using Image J.

2.7 Water Vapor Transmission Rate/WVTR

Calcium chloride was placed in a test tube and the mouth was sealed with the film sample. The tube was then placed in a desiccator containing saturated sodium chloride salt solution at an RH of 75%. Subsequently, it was weighed for 3.5 hours with an interval of 30 minutes. The increase in the mass of the tube was then plotted as a function of time. WVTR calculation was carried out using the formula below:

$$\mathrm{WVTR} = \frac{\mathrm{Slope}}{\mathrm{A}} \tag{1}$$

Description:

WVTR =Water vapor transmission rate (g/m^2/hour)
Slope =Change in weight per unit time (g/hour)
A =Film area (m^2)

2.8 Compressive Strength (Ulyarti et al., 2020)

Instrumentation LFRA Texture Analyzer Brookfield brand was used to measure the compressive strength with a probe-type TA 7 60 mm. The cycle count test was then carried out with 2 g trigger, 2 mm distance, and 2 mm/s speed. The size of the edible film sample to be tested was 5×2 cm.

2.9 Film Thickness

The samples were measured using a micrometer at 5 different places. The thickness of the edible film was expressed in terms of the average thickness.

2.10 Transparency (Piñeros-Hernandez et al., 2017)

The film was cut into squares with a size of 50×10 mm and placed in a spectrophotometer cell. The %transmittance was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm. The transparency of the edible film was then calculated with the formula below:

$$\mathrm{Transparency} = \frac{\log \%\mathrm{T}}{\mathrm{Thickness}\ (\%/\mathrm{mm})} \tag{2}$$

2.11 Solubility

The film sample was cut into sizes of 2×2 cm, placed in 50 mL of water, and then soaked for 24 hours with periodical stirring. The solution was filtered using a filter paper of unknown weight. Subsequently, the paper and residue were dried at 105°C for 24 hours, and the amount of undissolved film was weighed. The data obtained were then used to calculate the %solubility of the edible film in water.

2.12 Statistical Analysis

Statistical analysis obtained from 4 replicates was done by ANOVA. Duncan's new Multiple Range test at a 5% significance level was applied to find out significant differences in mean.

3. RESULT AND DISCUSSION

3.1 Native and Modified Cassava Starch

The yield of modified cassava starch produced in this study was 83.55%. The granules of native cassava starch are round, elliptical to oval shape with a smooth surface as shown in Figure 1a. Meanwhile, the granule of the modified form experienced shrinkage and folding with a rough surface, as shown in Figure 1b. During the modification process, gelatinization led to the dissolution of some starch components, which damaged the granule structure. Starch granules are composed of two types of polysaccharide called amylose and amylopectin. These molecules form amorphous and crystalline lamellae in the granule (LeCorre et al., 2011). The amorphous area is more susceptible to hydrolysis than the crystalline area. In yam starch, the granules exhibit semi-crystalline structure (Nadia et al., 2014) in which the center of the granules is mainly composed of amorphous structure while the crystalline present in the outer layer (Wang et al., 2008). During modification of starch in the present study, along with a strong destruction level in the center of granules, it can be seen that a lower destruction level of starch granules also occurred in the granule surface shown by the rough surface as seen in Figure 1b.

The distribution size of both native and the modified cassava starch in Table 1 shows that the modification disintegrates the granules and disrupts the inter and intramolecular bonding within the granules leading to smaller sizes of particles. These small particles formed aggregates as seen in Figure 1b.

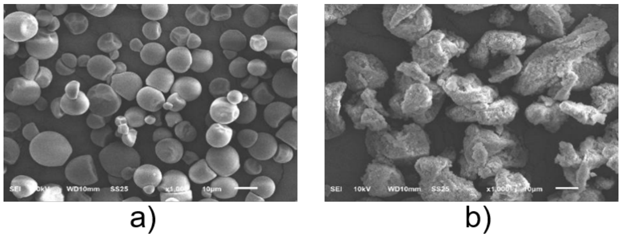
a) b)

Figure 1. Native Cassava Starch Granules (a) and Modified Cassava Starch Granules Precipitation Method (b)

Table 1. The Distribution Size of Starch Particles

Size (μm)	Native	Modified
< 10	114	206
10 - 19.99	54	187
20 - 29.99	11	74
30 - 39.99	10	36
40 - 49.99	7	19
50 - 59.99	4	16
60 - 69.99	7	13
70 - 79.99	3	7
80 - 89.99	2	2
90 - 99.99	3	6
>100	45	38
Total	260	604

3.2 Edible Film Morphology

The edible film morphology can be observed using a scanning electron microscope (SEM) on the surface and cross-section. SEM analysis on the surface showed that the higher the concentration of modified starch added, the rougher the appearance, as illustrated in Figure 2. The surface of the film without modified starch (2a) was smoother and had very few visible solids compared to the other films, which were slightly wavy (2b-e). These rough surfaces of the films indicate that they were less homogeneous. The cross-section of the edible films shows an increasing thickness of the film. The film without modified starch has a lot of small empty spaces. Only little empty spaces are seen with increasing modified starch concentration but bigger. Overall, the cross section of film with increasing modified starch concentration has finer cross section indicating a slightly denser film, as shown in Figure 2A-E.

3.3 FTIR Spectra

The FTIR spectra of the 2 starch types showed that the modification process did not change the functional groups present in them (Figure 3). However, there were differences in the intensity of the hydroxyl group transmission and bond vibration with water, at 3,000–3,600 cm^{-1} and 1,600 cm^{-1}. The broader modified starch peak at 3,000–3,600 cm^{-1} indicated an extension of hydrogen bond formation (Orsuwan and Sothornvit, 2017). As the intermolecular bonds in the amorphous area were broken, new bonds were formed between the modified granules and water available during modification through hydrogen bonding. The sharper peak at 1635 cm^{-1} on the modified starch spectrum represented a more bound water (Ulyarti et al., 2022). The FTIR spectra of edible films with 0%, 5%, and 10% modified starch was similar to that of normal starch, except the signal loss at a wave number of 2,931 cm^{-1} which corresponds to stretching vibration of C–H (Agi et al., 2019). Concentrations above 10% produced films with a weaker and missing functional group signal.

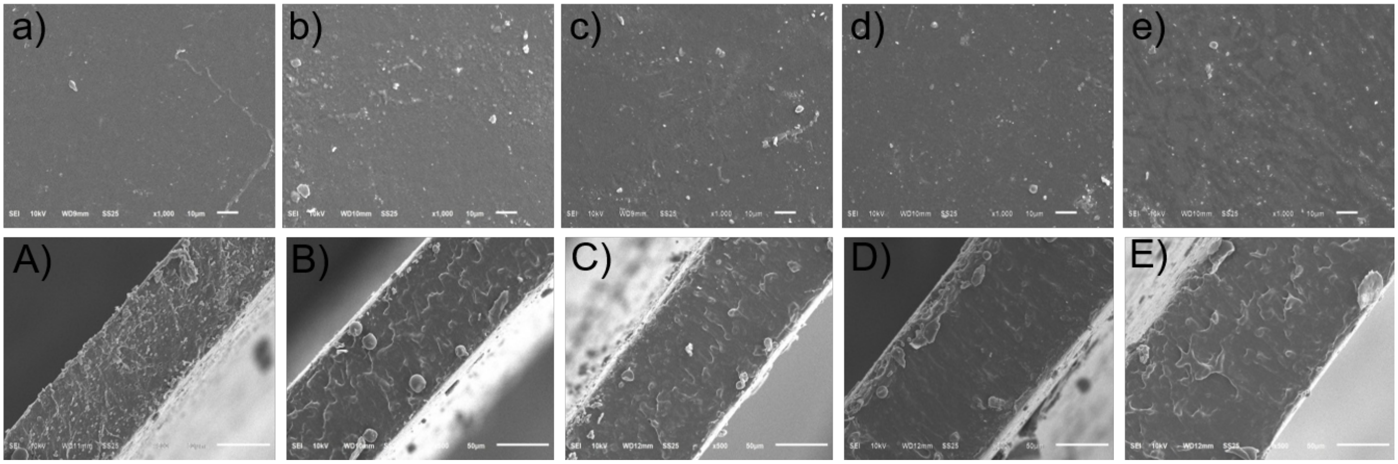

Figure 2. SEM Image of Surface and Cross-Section of Edible Film with Various Concentrations of Modified Starch at 500× Magnification. Film Surface 0% (A), 5% (B), 10% (C), 15% (D), and 20% (E), Film Cross-Section 0% (A), 5% (B), 10% (C), 15% (D), and 20% (E)

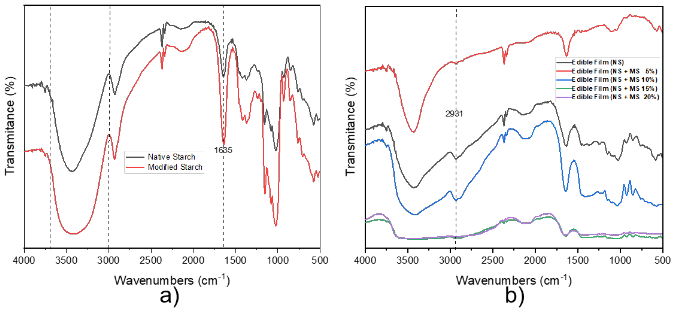

Figure 3. FTIR Spectra of Starch (a) and Edible Film (b) Where NS: Native Starch, MS: Modified Starch

3.4 The Characteristics of the Edible Film with the Addition of Modified Starch

The addition of modified starch tends to decrease the WVTR of the edible film, but its con-centration has no statistically significant effect (p<5%), as shown in Figure 4. The water vapor transmission rate is one of the most important parameters in assessing the quality of edible films. WVTR is the velocity of water vapor passing through a unit area of film during a certain unit of time. It is strongly influenced by RH, aw, temperature, plasticizer concentration, edible film-forming properties, and the type of material used. The results showed that WVTR of the edible film starch decreased to 21.00 g/m^2.hour after the addition of modified starch. This finding indicates that concentrations of ≤20% tend to inhibit WVTR. Similar results were also obtained in previous studies where an increase in concentration led to a slight decrease in WVTR (Farrag et al., 2018; Ulyarti et al., 2020). The values obtained in this study are lower than those reported for cassava starch based edible films where an average of 32.6 g/m^2.hour to 62.4 g/m^2.hour was recorded after the addition of gelatin (Ulyarti et al., 2020) and 25.36 to 30.35 g/m^2.s for starch/chitosan and starch/gelatin film (Silva et al., 2019). Although less homogeneous, the denser the cross-section of the film with higher the concen-tration of modified starch is one reason for lower WVTR of the film. Furthermore, the FTIR result confirms the changes on the microstructure of the starch film upon modified starch presence at concentration higher than 10%.

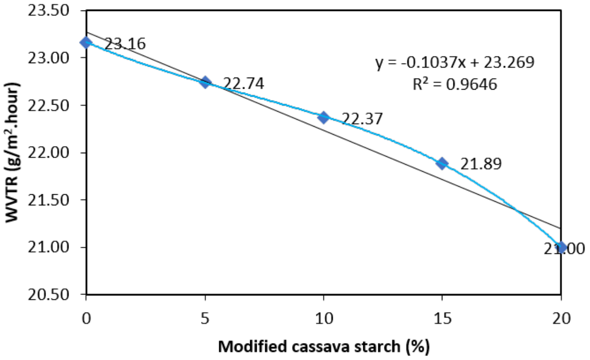

Figure 4. WVTR Edible Film at Several Levels of Modified Starch Concentration

Table 2 shows that the modified starch concentration had a significant effect (p<5%) on the compressive strength, thickness, transparency and the solubility of the films. Compressive strength is a mechanical property of edible film as well as a parameter that determines the ability to withstand loads. It also affects the ability of the material to with-stand pressure when applied at the maximum limit. Table 1 shows that the addition of modified starch in the manufacture of edible films significantly increased the compressive value by strengthening its matrix (p<5%). The value obtained also affects the ability of the film to protect the product. High compressive strength is

Table 2. The Characteristics of the Edible Film with the Addition of Modified Starch

Modified Starch Concentration (%)	Compressive Strength (N/m^2)	Thickness (mm)	Transparency (%)	Solubility (%)
0	56.18^{a}±0.15	0.18^{a}±0.002	9.922^{d}±0.51	75.10^{e}±1.28
5	66.30^{b}±0.11	0.19^{b}±0.001	9.419^{c}±0.44	69.72^{d}±1.90
10	73.46^{c}±0.29	0.20^{c}±0.001	9.286^{c}±0.20	63.16^{c}±2.32
15	80.05^{d}±0.52	0.21^{d}±0.005	8.776^{b}±0.22	57.43^{b}±4.80
20	89.45^{e}±0.15	0.22^{e}±0.003	6.484^{a}±0.14	52.19^{a}±3.62

Description: The numbers followed by the same lowercase letters in the same column are not significantly different according to the DNMRT test at the 5% level

needed for food product packaging to protect them during the handling, transportation, and marketing processes.

The compressive strength value recorded for cassava starch edible film in this study is higher compared to another study with 27.66 N/m^2 to 37.60 N/m^2 for cassava starch edible film with addition of gelatin (Ulyarti et al., 2020). The development of dense film structure as seen in SEM images (Huntrakul et al., 2020; Piñeros-Hernandez et al., 2017), less hydrogen bond formation and less bound water in film as indicated by FTIR (Ulyarti et al., 2022; Orsuwan and Sothornvit, 2017) may explain this.

Thickness is a physical property of edible film, which is influenced by the concentration of dissolved solids in the film solution as well as the weight of the film solution per unit area of the mold used. Film thickness is an important parameter that affects its application as a material for product packaging. It also affects other parameters, such as transparency, tensile strength, and the transparency rate of steam or gas on the material (González et al., 2015; Gujral et al., 2021; Hakke et al., 2022; Totosaus et al., 2020). The results showed that increasing the concentration of modified cassava starch significantly increased the thickness of the edible film ($p<5\%$), as shown in Table 2. The similar result was reported for potato starch-based nanocomposite film (Gujral et al., 2021).

Transparency of the edible film, if applied for food packaging, directly influences the consumer's acceptance. The transparency was measured by the amount of light it can transmit. The values obtained in this study are presented in Table 1. The analysis of variance showed that the modified starch concentration had a significant effect ($p<5\%$) on the transparency of the film, and it decreased with increasing modified starch concentration. This result is in line with that of a previous study where there was also a decrease in the transparency of composite edible films produced from zein (corn protein) nanoparticles and corn starch (Zhang and Zhao, 2017). The addition of materials, such as modified starch increases the total dissolved solids, which together with the compactness and homogeneity, greatly affects the level of transparency (Alves et al., 2015). In this study, the amount of total dissolved solid was kept constant, but there is an increase in the percentage of modified starch leading to a less homogeneity film as seen in SEM images of film surface and so the transparency.

The solubility of edible films is one of the important factors used in determining film quality. It is also used to determine their designation, for example, sausage products require films with high solubility and low transmission rates, which makes them easy to consume and not easily oxidized. The values of film solubility obtained in this study are presented in Table 1. The variance analysis showed that the addition of modified starch had a significant effect ($p<5\%$) on the solubility of the edible film. There was a decrease in the solubility with increasing modified starch. This result is in line with the decreasing in WVTR and the missing in functional groups detected by FTIR for hydrogen bonding and bound water. Similar results had been reported that decreasing of both WVTR and solubility occur simultaneously (Pérez-Vergara et al., 2020).

4. CONCLUSION

The concentration of modified starch has a significant effect on the compressive strength, thickness, transparency, and solubility of edible films. Although statistically insignificant, the increase in modified starch concentration tends to decrease WVTR of the film. The best film produced with 20% modified cassava starch, containing 3.2 g native starch, 0.8 g modified starch, 3 g glycerol, and 143 g distilled water, has 21.00 g/m^2.hour WVTR, 89.45 N/m^2 compressive strength, 0.22 mm thickness, 6.484%/mm transparency, and 52.19% solubility.

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