Acetylated sago starch-based antimicrobial edible film

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Abstract. Sago starch has potential to be used as the material of edible film packaging. However, the use of native starch has a limitation due to the hydrophilic character. Modified sago starch with acetylation can improve the characteristics of native starch. Most of the food contamination can be found in the surface area of the product. Antimicrobial compounds such as chitosan or garlic extract can be added to the edible film so it can protect the product from contamination. The experiments investigated the effect of acetate sago starch (3, 4, 5%) on the physical and mechanical properties of edible film. From the best formulation of edible film-making, the antimicrobial compounds were added. There were two antimicrobial compounds, garlic extract and chitosan; and three levels of chitosan concentration (0; 15; and 30%) and garlic extract (0; 0.2; and 0.4%). Each antimicrobial activity was carried out by in vitro assay using the disk diffusion method (diameter 5 mm). The best edible film resulted from 4% starch acetate with high tensile strength (1.635 MPa) and elongation (49.101%) values. The edible film with the addition of 0.4% garlic extract has the highest inhibitory activity against Escherichia coli (21.40 mm) and against Salmonella typhi (28.20 mm).

Keywords: acetylated starch, antimicrobial activity, edible film, physical-mechanical properties, sago starch

1. Introduction
Indonesia has many types of starch-producing plants that have potential to be developed. Sago (Metroxylon sp.) is a tropical plant that grows in Indonesia and has the potential as a source of starch. The results of the study showed that more than 50% of the world's sago population is spread across Indonesia with an area of around 4,700,000 ha so that the utilization of sago starch is very potential [1]. One of the great potentials of sago starch application is as plastic or biodegradable packaging material [2]. One type of biodegradable packaging is edible film that can be used to coat and protect food. The film forming ability is related to the amylose content. High amylose content will result in a better film forming ability [3]. Sago starch has high amylose content (28.84%) [4], and it is potentially used as edible film material. However, the unmodified starch has a limitation due to its friability when used as edible film material. [5]. Several modifications are carried out to produce more hydrophobic starch including acetylation. Acetylation is a chemical process to produce starch acetate by substituting acetyl groups into starch molecules [6].

Antimicrobial compounds can be added to edible films to protect the product from contamination and increase the shelf life of the product [7]. Chitosan is a natural polysaccharide derived from chitin
and has antimicrobial activity, that is most effective against yeast, mold, gram-positive bacteria, and gram-negative bacteria [8]. Besides chitosan, the use of essential oils can be used as an alternative to artificial preservatives. An essential oil that has high antimicrobial benefits is garlic [9]. This work focuses on the suitability acetylated sago starch as materials for making edible films, with the best physico-chemical properties. The aims of this research were to investigate the effect of sago acetylated starch (3, 4, 5%) to the edible film properties, and to investigate antimicrobial activity of edible film.

2. Material and methods

2.1. Material
Sago starch (*Metroxylon* sp.), acetate starch, distilled water, glycerol, chitosan, and garlic.

2.2. Preparation of acetate starch
50 g of native starch were suspended in 250 ml of distilled water, shaken for 30 minutes and pH was adjusted to 8. Then 4.2 g of acetic acid anhydride were slowly added and pH was maintained at 8-8.4. The acetylation was reacted at 30 °C for 10 minutes, then the pH was adjusted to 4.5. The suspend were washed with distilled water three times, and dried at 45 °C for 24 hours.

2.3. Preparation of starch edible film
A suspension of native and acetate (3, 4, and 5%) was mixed with glycerol (40%, V/W) at room temperature (25 °C) for 5 min. This suspension was transferred to a water bath at 90 °C for 30 min and agitated by magnetic stirrer (500 rpm). After cooling, about 50 mL of the sample was poured into casting tray and then dried at 60 °C in an oven to cast the films.

2.4. Physico-chemical characterization

2.4.1. Film thickness. Film thickness was measured using a micrometer. The final value represented the average of 5 measurements taken at different parts of the film.

2.4.2. Moisture content. Measurement of the water content of film used the oven method. A total of 2–10 g of the sample was weighed in a cup that was known for its weight (a), then dried in an oven at 105 °C for 5 hours. The sample is then cooled in a desiccator and weighed until the weight is constant (b).

\[
\text{Moisture content (\%) = } \frac{a-b}{a} \times 100\% \tag{1}
\]

2.4.3. Water vapor transmission rate. The film specimens to be tested are cut to an area of 10x10 mm. Then the place of silica gel in the desiccator is replaced with distilled water, and silica gel is put in a plastic jar (film testing place) as much as ¾ of the volume jar. Before that, silica gel was dried at 105 °C for a minimum of 3 hours. The film specimens which have been cut and then made into a jar lid, are stored in a desiccator containing distilled water. The weight of jar containing silica gel and covered film specimen is measured at 0, 1, 2, 3, 4, and 5 hours.

Water vapor transmission rate is calculated using the formula:

\[
\text{WVTR} = \frac{\Delta W}{t \times A} \tag{2}
\]

Where,
- \( W \) = change in weight of silica gel after 5 hours (g)
- \( t \) = time (5 hours)
- \( A \) = film surface area (mm²)
2.4.4. Transparency. The transparency value of the films was obtained by measuring its transmittance in a spectrophotometer, at a wavelength of 600 nm. Samples were tested in two replicates. [13]

2.4.5. Mechanical properties. The tensile strength and elongation at break were determined using (ASTM D 3039, 2012).

2.4.6. Solubility film. Samples with a size of 4 x 2 cm for each film were dried up to 40°C until reach constant weight (Wo), submerged in a beaker with 50 mL of distilled water, and the covered vessels staged at 27°C with some agitation for 24 h. After this time, samples were released and dried up to 40°C until reaching constant weight (W2). [14] Percentage of water solubility (% WS) was calculated as follow:

$$\text{WS} = \frac{(W_o - W_2)}{W_o} \times 100$$

(3)

2.5. Statistical analysis
Data were analyzed using randomized complete design with 2 replicates. Duncan test at a confidence level of 95% was used to observe differences among the edible film (3, 4, and 5% acetate starch).

2.6. Preparation of starch antimicrobial edible film
Antimicrobial edible films were prepared using starch acetate with selected concentration (4%). Antimicrobial compounds used are chitosan and garlic extract.

2.6.1. Preparation of starch-chitosan antimicrobial edible film. Chitosan (0, 15, 30%) is dissolved in 2% acetic acid. Next, starch acetate was dissolved in distilled water and stirred for 5 minutes at room temperature (25°C). The solution was heated to a temperature of 90°C for 15 minutes and then added chitosan dissolved in 2% acetic acid and stirred for 30 minutes. After that, the temperature is reduced to 70 or more, add glycerol 40% (w / w) and stir with a magnetic stirrer for 10 minutes. Then the heat is reduced to 40°C for 20 minutes [15].

2.6.2. Preparation of starch-garlic antimicrobial edible film. The starch is dissolved in 100 ml of distilled water at room temperature (25 °C). Next, the suspension is heated until 90°C for 15 minutes. The solution is mixed with glycerol 40% (w / w) and stirred with a magnetic stirrer (500 rpm) at 70°C for 10 minutes. Then the temperature is reduced to 40°C then the garlic extract (0, 0.2, 0.4%) is added and stirred for 20 minutes [16].

2.7. Antimicrobial activity
Antimicrobial activities were evaluated with disk diffusion method. 10 ml of liquid media is poured into a sterile petri disk. 0.1 ml of Escherichia coli and Salmonella typhi culture were put and spread into media. On the media, 2 holes were made with a diameter of ± 5 mm. Each hole is filled with 1 ml of edible film solution, then the media was incubated at 37°C for 2 x 24 hours. The clear zone that forms around the hole is the inhibition zone of the edible film and measured using a caliper (mm).

3. Results and discussion

3.1. Acetate starch
The acetylation process in this study was conducted to obtain acetate starch with a low DS value (0.01-0.2). Acetate starch with a degree of substitution can be used as film forming [17]. The acetylation process is carried out by mixing starch and acetic acid anhydride reagents. The acetate starch has an acetyl content of 2.30% and degree of substitution of 0.09. The acetylation process is influenced by several factors, such as reaction time, acetylation reagent concentration, temperature, and pH.
3.2. Edible film characterization

Characterization of edible films are shown in Table 1.

| Characteristic          | Native starch | Acetate starch 3% | Acetate starch 4% | Acetate starch 5% |
|-------------------------|---------------|-------------------|-------------------|-------------------|
| Moisture content (%)    | 10.435±0.182a | 10.100±0.018b     | 8.348±0.207c      | 6.186±0.012d      |
| Solubility (%)          | 36.352±0.412b | 39.507±0.334a     | 34.340.750c       | 26.145±0.169d     |
| Thickness (mm)          | 0.185±0.007a  | 0.125±0.007b      | 0.145±0.007b      | 0.175±0.007a      |
| Transparency (%T)       | 84.036±0.354  | 87.065±0.805      | 87.544±0.051      | 89.552±0.740      |
| Tensile strength (MPa)  | 0.814±0.035c  | 0.650±0.018c      | 1.635±0.033b      | 2.180±0.295a      |
| Elongation (%)          | 38.925±0.148e | 35.717±0.033d     | 49.101±0.001b     | 63.925±0.049a     |
| WVTR (g/jam.mm²)        | 1.165±0.228   | 1.186±0.006       | 0.876±0.008       | 0.771±0.028       |

The numbers on the same line followed by the same letter are not significantly different at the 5% test level.

In Table 5, it can be seen that the higher the percentage of sago starch used, the thicker the resulting edible film will be. The thickness value has increased due to the concentration of material increases, while the volume of the solution are the same [18]. The average solubility value of edible films is around 26.145-39.507%. The solubility value of starch acetate film is lower than its native. This is due to the increased hydrophobicity of starch due to the modification process of acetylation [13]. The value of moisture content decreases with increasing acetate concentration. This is due to the modification of acetylation increasing hydrophobic properties in starch by the substitution of acetyl groups into starch molecules [6].

Transparency plays an important role when edible film will be applied because it will affect the appearance of the product. On the Table 1, it can be seen the increase of the thickness of the film, so the transparency increases slightly. Edible films with 5% acetate starch has the highest transparency values. Starch increases water retention capacity thereby facilitating light exchange [14]. The average value of tensile strength of edible films is between 0.66-2.39 MPa. The acetate edible film has higher value than its native edible film. The acetylation process affects the hydrophobicity of starch so that the film is not brittle. The average value of elongation of edible films is between 28.2-56.48%. The average value of WVTR of edible films is between 0.771-1.186 g/jam.mm². The WVTR value of the acetate edible film is lower than the edible native starch edible film. That indicates that acetate edible film has a higher barrier of water.

The results of the analysis of the characteristics of edible film showed a difference between native edible film starch and acetate edible film starch. But the value of the water vapor transmission rate does not show any difference (F arithmetic <F table). Therefore, in the next step, antimicrobial edible films made with 4% acetate starch. Acetate starch (4%) produces edible films with better characteristics than native edible films, and relatively same with 5% acetate edible film.

3.3. Antimicrobial activity

Antimicrobial activity depends on several factors like the concentration of the extract, the content of antibacterial compounds, the diffusion power of the extract, and the types of bacteria that are inhibited. Antimicrobial activity of edible films containing chitosan and garlic extract was tested against Escherichia coli and Salmonella typhi where the microbes are primary contaminants in food products, especially in meat. Antimicrobial activity is measured by the clear zone surrounding the film. The clear zone is the zone of inhibition of the film against microbes. The antimicrobial activity of the edible film can be seen figure 1 and 2, while the average value of clear zone diameter is presented in Table 2.
Figure 1. Inhibition zone of edible film against *Salmonella typhi*.

Figure 2. Inhibition zone of edible film against *Escherichia coli*.
Table 2. Antimicrobial activity of edible film.

| Edible film | Average value of clear zone (mm) | Salmonella typhi | Escherichia coli |
|-------------|----------------------------------|------------------|------------------|
| K1          | 0.00 ± 0.00                      | 0.00 ± 0.00      |                  |
| K2          | 17.50 ± 0.71                     | 11.50 ± 0.71     |                  |
| K3          | 20.01 ± 0.01                     | 15.00 ± 1.41     |                  |
| K4          | 0.00 ± 0.00                      | 0.00 ± 0.00      |                  |
| K5          | 21.10 ± 0.14                     | 15.50 ± 0.70     |                  |
| K6          | 28.20 ± 1.41                     | 21.40 ± 0.28     |                  |

Notes: K1 = Starch+chitosan 0%  K4 = Starch+garlic 0%
K2 = Starch+chitosan 15%  K5 = Starch+garlic 0.2%
K3 = Starch+chitosan 30%  K6 = Starch+garlic 0.4%

Table 2 shows that the higher the concentration of chitosan and garlic extract, the higher the inhibitory activity of *Salmonella typhi* was. The higher the concentration of antimicrobial, the higher the antimicrobial content, therefore the inhibition microbial growth will increase. The highest inhibitory value of *Salmonella typhi* is found in 0.4% garlic extract with an average diameter of 28.20 mm. Inhibition of bacterial growth by garlic extract due to the presence of the most active compounds in garlic, allicin (allyl-propenethiosulphinate) and its derivatives (dialyl thiosulfinate and dialyl disulfide).

The result of antimicrobial activity of Edible film added with chitosan 15% has the lowest inhibition zone diameter of *Escherichia coli* which is 11.50 mm and edible film added with garlic 0.4% has the highest inhibitory activity (21.40 mm.). The difference in antimicrobial activity can be caused by several factors such as the concentration and characteristics of the active compound, also properties of the film material. Garlic extract has sulfide compounds against bacteria by destroying the bacterial cell wall structure, inhibiting enzymes in bacteria, and damaging the RNA synthesis process that stops bacterial growth.

The results of antimicrobial activity testing showed that the biggest inhibition was garlic extract 0.4% and chitosan of 30%. The mechanism of chitosan in inhibiting microbial activity is the presence of amino groups attached to the cell membrane through electrostatic interactions.

4. Conclusion
Edible film with 4% acetate sago starch has high average tensile strength value of 1.635 MPa and an average elongation value of 49.101%. Furthermore, edible film with 4% starch acetate has a low moisture content of 8.348% and a WVTR value of 0.876 g / hour.mm². Edible film with the addition of chitosan and garlic extract has antimicrobial activity against *Salmonella typhi* and *Escherichia coli*. Edible film with the addition of 0.4% garlic extract has the highest inhibitory activity against *Escherichia coli* (21.40 mm) and against *Salmonella typhi* (28.20 mm).

5. References
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Acknowledgments

We would like to thank the Beasiswa Unggulan, Ministry of Education and Culture and the Department of Agricultural Industry Technology for financial assistance during the research.