

Biofilm Based on Modified Sago Starch: Preparation and Characterization

Dewi Sondari ^{1*}, Faizatul Falah ¹, Riska Suryaningrum ¹, Fahriya Puspita Sari ¹,
Athanasia Amanda Septefani ², Witta Kartika Restu ², and Yulianti Sampora ²

¹ Technology Process of biomass and bioremediation Group, Research Center for Biomaterial, Indonesian Institutes of Sciences (LIPI), Kawasan Cibinong Science Center, Jl. Raya Bogor Km.46, Cibinong, West Java, Indonesia 16911

² Polymer Chemistry Group, Research Center for Chemistry, Indonesian Institutes of Sciences (LIPI), Kawasan Puspiptek Serpong, South Tangerang, Banten, Indonesia 15314

*) Corresponding author: sondaridewi@gmail.com

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Abstract

Biofilms made from modified sago starch to improve the mechanical and physical properties have been studied. This study aimed to prepare and characterize biofilms from sago starch and modified sago (acetylation, oxidation, cross-link and precipitation). Modified of sago starch was prepared by some methods: precipitation using ethanol as solvent, acetylation modified of sago starch using acetic anhydride, oxidation modification using hydrogen peroxide and cross-link modification using sodium acetate. Biofilms were made from modified sago using glycerol with a concentration of 1.0% weight, where glycerol acts as a plasticizer to impart the thermoplasticity of the starch film. Biofilm made from native sago was then compared to biofilm from modified sago starch. The effects of modified sago starch to the biofilm were measured on its moisture contents, contact angle value, mechanical properties such as elongation and tensile strength. The chemical structures were evaluated by Fourier-transform infrared spectroscopy (FTIR) and morphology of biofilm were measured by Scanning Electron Microscope (SEM). The optimum condition of modified sago starch used in biofilm in this study is by acetylation. The result show that modified acetylation of sago starch can improve the properties of biofilm

Keywords: *biofilms; sago; acetylation; oxidation; cross link; SEM; FTIR*

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INTRODUCTION

Biofilms are produced from renewables, such as polysaccharides, proteins, and lipids or a mixture of them. They are used as coatings in packaging industries

to reduce the serious environmental concerns by minimizing the usage of plastic packagings (Thakur *et al.* 2016). The biofilms can be added with functional ingredients, such as antimicrobial and antioxidant

agents, nutraceuticals, or color and flavor ingredients. Glycerol as a plasticizer, gives the pliability and flexibility to improved handling on making starch-based films and coatings (Krishna Das *et al.*, 2013).

Biopolymers such as polysaccharides, proteins, and lipids can be used for the formation of biofilms and coatings suitable for food and nonfood packaging applications, they can reduce traditional polymeric packaging such as plastics. Polysaccharides like starch, is used in biofilms and coatings because of its good mechanical properties. Lipids like beeswax, mineral oil, vegetable oil, surfactants, acetylated monoglycerides, carnauba wax and paraffin wax strongly affect the permeability of films and coatings (Kafrani *et al.*, 2016).

Starch-based films have been particularly considered for the reason that they show physical characteristics similar to synthetic polymers: transparent, odorless, tasteless, semi-permeable to CO₂ and resistant to O₂ passage. From the literature, it is found that most of the researchers studied the development of biodegradable films using pure starch that are very brittle in nature as a result of the strong cohesive bond between the polymer molecules (Bertuzzi *et al.*, 2007; Chang *et al.*, 2006; Flores *et al.*, 2007; Lu *et al.*, 2009; Zhang & Han, 2006).

Plasticizer are needed to reduce the brittleness and enhance the flexibility of the biofilms. Glycerol is the most widely used plasticizer to improve the mechanical properties and transparency of the biofilms. The hydroxyl groups that present in glycerol are responsible for inter and intramolecular interactions (hydrogen bonds) in polymeric chains, providing films with a more flexible structure (Souza *et al.*, 2012).

Starch modification can be divided into four classes namely: physical, chemical, enzymic and biological modifications. Among these modification methods, chemical means is the most frequently used process (Daramola and Osanyinlusi, 2006). Chemical modifications of starch involves the treatment of native starch with specific chemicals reagent. Those modifications are acetylated, oxidized, lintnerized, pyrodextrinized, hydroxypropylated and cross-linked starches (Kaur *et al.*, 2004).

Sago starch had some disadvantage such as inconsistent viscosity, varied pasta texture, mouldy odor, and slight reddish color. It also had some limitations in usage due to its resistance to shear and low temperatures, high retrogradation and syneresis, low heat resistance and solubility, and also its instability in acidic environment. Due to those limitations and disadvantages, some modifications of sago starch are needed to improve its physical and mechanical properties (Mirmoghtadaie *et al.*, 2009; Cock, 1982; Miyazaki *et al.*, 2006).

The present work was focused on the biofilm produced from modified sago starch. The characteristics of native sago and modified sago to enhance the mechanical properties, moisture content, FTIR, SEM and hydrophobicity have been studied.

MATERIALS AND METHODS

Material

Sago, sodium hydroxide, sodium acetate, silicon oxide, amylose, potassium sulphate, indicator, acetic anhydride, hydrogen peroxide, ethanol (Merck, Darmstadt, Germany). Glycerol, petri dish, furnace, oven, dessicator, soxhlet apparatus, spectrophotometer UV-Vis instruments equipment, SEM HITACHI SU3500, contact angle meter model CAM-PLUS MICRO (Tantec Inc., USA).

Methods

Modified of sago starch

Oxidation modification of Sago starch with hydrogen Peroxide: Pure sago starch is oxidized using 1% concentrates of hydrogen peroxide according to the method carried out by Catarina *et al* (2016).

Acetylation was performed according to previous researcher (Pietrzyk *et al.*, 2018). 200 g of starch (native) were dispersed in 460 g of distilled water. Then, acetic anhydride was added drop-wise to the stirred starch suspension at pH kept between 8 and 9 by adding 2% NaOH solution. When the whole amount of acetic anhydride was added, the suspension was further stirred for 15 min. After the reaction, the modified starch was washed with distilled water, dried at a temperature of 20°C for 48 h.

Sago starch is added with distilled water and silicon oxide is stirred for 5 minutes, then sodium hydroxide solution is added and stirred for 20 minutes. After that the sodium acetate cross link agent is added with a variation of 10%, 15%, 20% and 25% of the weight of starch and stirred for 15 minutes, then heated in a water bath at 400 °C for 1 hour, then filtered and dried in an oven at 500 °C for 3 hours (Akpa *et al.*, 2012).

Sago starch (8 g) was dispersed in 150 mL of distilled water and stirred vigorously for 1 h before the starch fully gelatinized. The solution was cooled down in room temperature then 150 ml ethanol was added dropwise for 5 h. The solution was centrifuged at 8000 rpm for 20 min and washed two times with ethanol. The supernatant was cast in the petri dish and oven dried overnight (Ma *et al.*, 2008).

Biofilm preparation

The preparation of biofilm was conducted using pure sago and modified sago. The sample was weighed 5 grams and then mixed with 100 mL of distilled water. Then stirred for 30 minutes at a temperature of 60 °C. Glycerol was added as a plasticizer with a concentration of 1% (v/v). 10 grams of sample was poured on a 100 mm diameter petri dish. The sample was dried overnight at a temperature of 45 °C (Moreno *et al.*, 2017).

RESULTS AND DISCUSSIONS

The chemicals structure can be investigated from the change of functional group by means of the absorption peak characteristic. Figure 1, 2 and 3 shows

Table 1. FTIR characteristic of native and modified of sago

Native sago	Samples (cm ⁻¹)			Characteristics
	Acetylation	Oxidation	Cross link	
3099-3585	3071-3567	3090-3576	3049-3561	OH groups
2087-2880	2071-2939	2072-2900	2088-2890	CH (aliphatic and aromatic)
1720-1725	1647-1655	1648-1654	1649-1658	C=O
1406-1413	1419-1427	1433-1439	1406-1432	CH ₃ antisym deformation
1325-1367	1325-1345	1330-1354	1313-1357	OH bending and CH bending
1139-1159	1151-1168	1150-1161	1150-1153	C-O-C, C-C stretching
930-1007	1000-1064	992-1083	996-1067	Isotactic band

the FTIR spectrums of native sago/acetylated modified, native sago/cross link modified, and native sago/oxidized modified respectively. The comparison of each absorption peak from the designated samples is summarized in Table 1.

The FTIR spectra of native sago/ modified acetylation of sago showed absorption peak at wavenumber around 3071-3567 cm⁻¹ attributed to the OH stretching and 2071-2939 cm⁻¹ correspond to CH stretchings of all samples, while the peaks at 1325-1367 cm⁻¹ correspond to (OH) and (CH) bendings were observed of the native sago starch. There was a small peak near at 1647 cm⁻¹ that was attributed to the stretching of the ester carbonyl C=O and indicated the acetylation of starch (Mano *et al.*, 2003).

In the spectrum for native sago/ modified cross link of sago, the presence of -C-O-C was confirmed by the absorption peaks at 1150-1153 cm⁻¹, the absorption peak at 1648 cm⁻¹ was attributed to C=O bonding. The

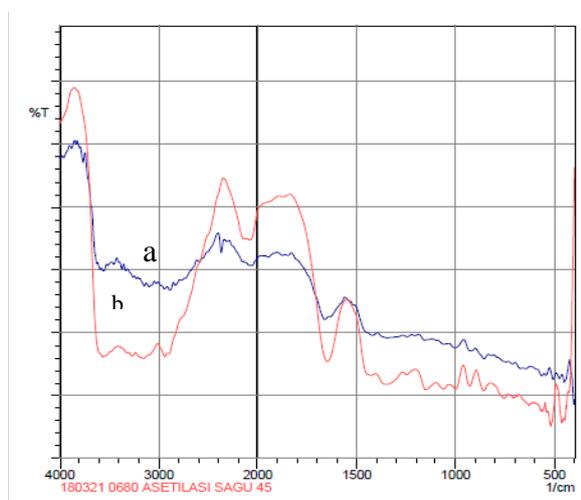


Figure 1. FTIR spectra of native sago (a) and modified acetylation of sago (b)

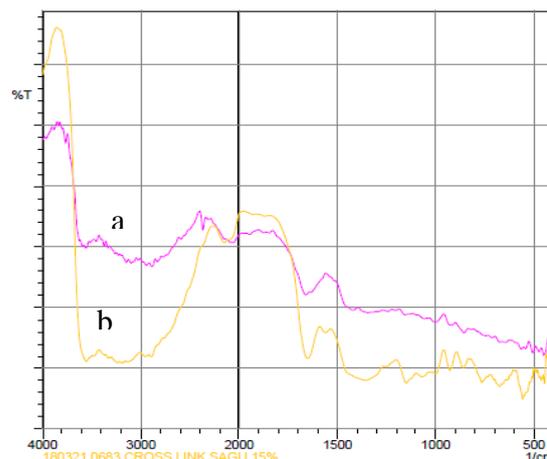


Figure 2. FTIR spectra of native sago (a) and modified cross link of sago (b)

comparison of FTIR spectra between native sago, modified acetylation of sago, modified cross link of sago and modified oxidation of sago showed there was no significant peak change, but difference in intensity of the peak. Where the intensity of vibration peak of C=O cm⁻¹ from modified acetylation of sago, modified cross link of sago and modified oxidation of sago at 1647-1658, cm⁻¹ were higher than native of sago. This indicated that modification of sago was occurred.

Native sago, modified of sago and their films were characterized their moisture contents and contact angles. Table 2 shows that the moisture content of biofilm from native sago starch is higher than that of modified one. The moisture content of the film affects the ability to absorb water in a film. The high moisture content decreases the quality of the film into a shorter service life due to bacterial interference (Pelissari *et al.*, 2013).

Water content is influenced by the hydrophobicity of the biofilm, the more hydrophobic the lower the water content. From this research, it can

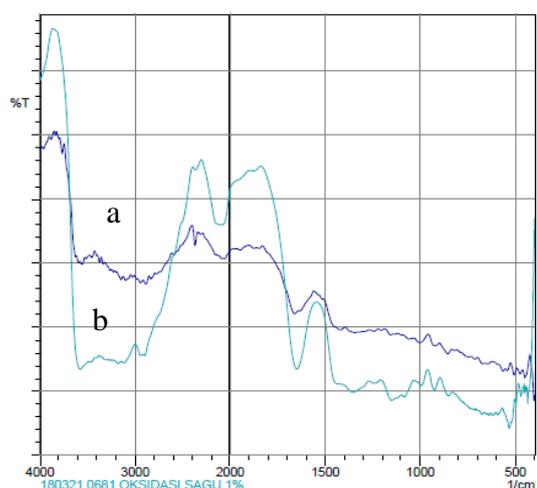


Figure 3. FTIR spectra of native sago (a), modified oxidation of sago (b)

Table 2. Moisture content and contact angle of starch film

Samples	Moisture content (%)	Contact angel
Native sago	0.26	27.43±3.06
Modified of sago :		
(1) Acetylation	0.18	60.41±1.31
(2) Oxidation	0.23	45.47±2.95
(3) Cross link	0.20	53.96±7.20
(4) Precipitation	0.22	47.68±5.17

be seen that the modification of sago by acetylation has the lowest water content of 0.18%, and the value of the contact angle is greater, so that the modification of sago by acetylation has high hydrophobicity properties.

The quality of biofilm can be seen from the hydrophilic nature of a film that can be determined using contact angles testing. The contact angles (CA) is the parameter used to estimate the film's resistance to water, and shows the hydrophobicity of the film surface (Phan *et al.*, 2005). Biofilms from sago starch modified their cross links, acetylation, oxidation, precipitation have higher contact angles than natural sago starch biofilms. The results obtained showed that the hydrophilic properties of sago starch correlated with the contact angle value. The high contact angle value of modified sago starch caused a decrease in the hydrophilic properties of modified sago starch films and improved hydrophobic properties. Likewise, the opposite is true of pure sago starch. The hydrophobicity of biofilm from modified sago starch results in better film quality because it increases the permeability to water and high oxygen so that food products coated with biofilm have a long shelf life, are not easily damaged by fungus or microorganisms due to environmental factors and good quality used as a food coating material (Balessterosa *et al.*, 2018).

Thickness affects the use of film on the product coatings because it will affect the rate of transmission of steam, gas, and volatile compounds as well as other physical properties such as tensile strength and elongation at break of the resulting biofilm. According to McHugh and Kroctha (1993) and Skurtys *et al.*, (2006), with the increasing of thickness (<0.250 mm), the better the ability to hold biofilm, resulting in longer shelf life of the product. Biofilm produced in this

Table 3. Mechanical properties of starch films

Samples	Tensile strength (MPa)	Elongation at break (%)	Thick ness (mm)
Native sago	9.04±2.04	12.32±6.04	0.10
Modified of sago:			
Acetylation	16.35±0.26	28.20±1.64	0.13
Oxidation	13.38±0.17	12.56±6.24	0.11
Cross link	14.42±0.08	13.35±2.40	0.12
recipitation	12.27±0.29	21.69±3.17	0.12

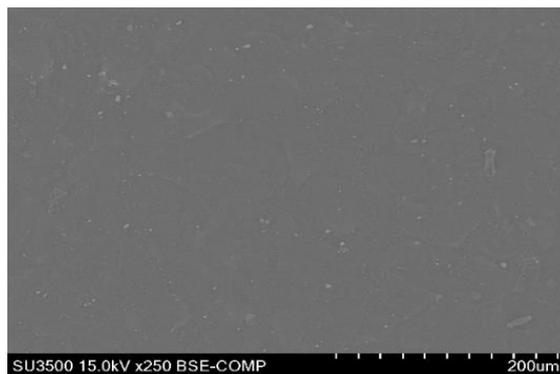


Figure 4. Morphology of biofilm from modified acetylation of sago starch

study has a thickness that has met the standard of the thickness of film.

The mechanical properties of the films are shown in Table 3, which demonstrated the value of tensile strength and percentage of elongation at break at 25°C. Tensile strength is the maximum stretch that can be received by the film until it breaks. The tensile strength of the biofilm analyzed ranged from 12-16 MPa. The presence of cross bonds can increase the interaction between amylose molecules to form hydrogen bonds more strongly, so that the film matrix will become thicker, denser, and stiffer. This study is in line with Atichokudomchai *et al.*, (2003) that the tensile strength of biofilm is influenced by the rigidity of the film and the strength of hydrogen bonds between amylose molecules so that the amylose structure formed is similar to the hydrophobic cyclodextrin.

Interaction between amylose molecules causes biofilm to be denser and stiffer so that the elongation level of biofilm is not too large. The results of the biofilm elongation test on sago modification by acetylation give a higher value of elongation at break compared to other modifications.

From Figure 4 and 5. it appears that the morphology of the biofilm from pure natural sago starch and sago starch modified by the acetylation method is not much different, which shows a smooth surface. Biofilm of pure starch and homogeneous modification without pores or cracks and starch molecules have been well dispersed (Garg and Jana, 2007).

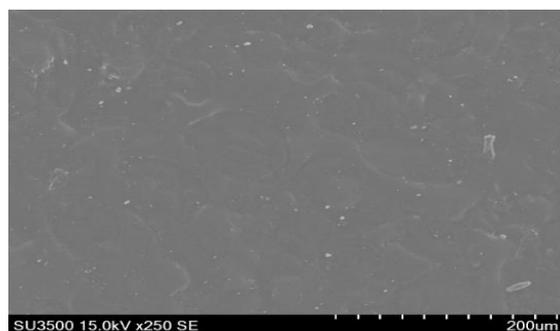


Figure 5. Morphology of biofilm from native sago starch

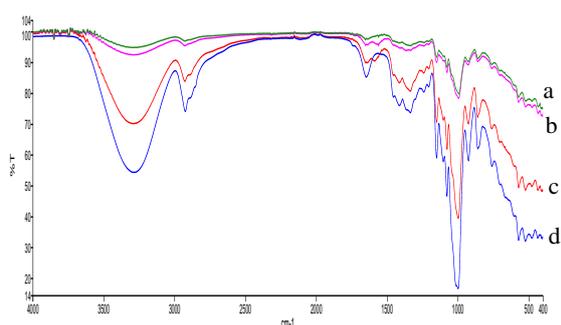


Figure 6. FTIR spectra of biofilm from modified sago (a) oxidation, (b) acetylation, (c) cross-link, (d) precipitation

The infrared spectra of biofilm from modified starch by oxidation, acetylation, cross link and precipitation are presented in Fig. 6. In the spectrum for starch biofilms, the broadband at $3284\text{--}3322\text{ cm}^{-1}$ was the OH stretching. The peak at $2927\text{--}2933\text{ cm}^{-1}$ was corresponding to the C–H stretching, while the bands at 1650 cm^{-1} for C=O bonding. Then, the bands from 985 to 1000 cm^{-1} was corresponding to the C–O–C complex.

CONCLUSIONS

The optimum condition of modified sago starch used in biofilm in this study is acetylation, it can improve the properties of the biofilm. High contact angle of modified sago starch (acetylation) is a desirable characteristic of edible coatings because the film is hydrophobic, so that when used as a food coating it will increase the shelf life and inhibit microbial growth.

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