Biofilm Based on Modified Sago Starch: Preparation and Characterization

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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 (Kafiani 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, linterized, 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 (Mirmoghaddaie 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 (Tantech 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
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 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
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 Krochta (1993) and Skurty 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 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).

| Samples          | Moisture content (%) | Contact angle
|------------------|----------------------|-----------------|
| Native sago      | 0.26                 | 27.43±3.06      |
| Modified 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      |

Table 3. Mechanical properties of starch films

| Samples          | Tensile strength (MPa) | Elongation at break (%) | Thickness (mm) |
|------------------|------------------------|-------------------------|----------------|
| Native sago      | 9.04±2.04              | 12.32±6.04              | 0.10           |
| Modified 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           |
| Precipitation    | 12.27±0.29             | 21.69±3.17              | 0.12           |

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

Figure 5. Morphology of biofilm from native sago starch
The infrared spectra of biofilm from modified sago starch by oxidation, acetylation, cross link and precipitation are presented in Fig. 6. In the spectrum for starch biofilms, the broadband at 3284-3322 cm$^{-1}$ was the OH stretching. The peak at 2927-2933 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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REFERENCES

Akpa, Gunorubon, J., Dagde, Kekpugile, K., (2012), Modification of cassava starch for industrial use, International Journal Engineering & Technology, 2, pp. 913-919.

Atichokudomchai, N., and Varavinit, S., (2003), Characterization and utilization of acid modified cross linked tapioca starch in pharmaceutical tablets, Carbohydrate Polymers, (53), pp. 263-270

Bertuzzi, M. A., Castro Vidaurre, E. F., Armada, M., and Gottifredi, J. C. (2007), Water vapor permeability of edible starch based films, Journal of Food Engineering, 80(3), pp. 972–978.

Catarina, S., Kumoro, A., Ratnawati, R., dan Retnowati, S., (2016), Modifikasi pati sukun (artocarpus altillis) dengan teknik oksidasi menggunakan hidrogen peroksida tanpa katalis, Teknik, 37(1), pp. 32-40.

Chang, Y. P., Abd Karim, A., and Seow, C. C. (2006), Interactive plasticizing anti plasticizing effects of water and glycerol on the tensile properties of tapioca starch films, Food Hydrocolloids, 20(1), pp. 1–8.

Das, D.K., Dutta, H., and Mahanta, C.L., (2013), Development of a rice starch-based coating with antioxidant and microbe-barrier properties and study of its effect on tomatoes stored at room temperature LWT, Food Science and Technology, 50, pp. 272-278.

Flores, S., Famá, L., Rojas, A. M., Goyanes, S., and Gerschenson, L., (2007), Physical properties of tapioca–starch edible films: Influence of filmmaking and potassium sorbate, Food Research International, 40, pp. 257–265.

Garg, S., and Jana, A.K., (2007), Studies on the properties and characteristics of starch–LDPE blend films using cross-linked, glycerol modified, cross-linked and glycerol modified starch, European Polymer Journal, (43), pp. 3976-3987.

Kafani, E.T., Shekarchizadeh, H., and Behabadi, M.M., (2016), Development of edible films and coatings from alginates and carrageenans, Carbohydrate Polymers, 137, pp. 360–374.

Kaur, L., Singh, N., and Singh, J. (2004), Factors influencing the properties of hydroxypropylated potato starches, Carbohyd Polym, 55, 211-223.

Lu, D. R., Xiao, C. M., and Xu, S. J. (2009), Starch-based completely biodegradable polymer materials, Journal of Polymer Science Part C: Polymer Letters, 3, pp. 366–375.

Ma, X., Jian, R., Chang, P.R., and J. Yu, J., (2008), Fabrication and Characterization of Citric Acid-Modified Starch Nanoparticles / Plasticized-Starch Composites, pp. 3314–3320.

Mano, J.F., Koniarova, D., and Reis, R.L., (2003), Thermal properties of thermoplastic starch/synthetic polymer blends with potential biomedical applicability, Journal of Materials Science: Materials in Medicine 14, pp. 127-135.
McHugh, T., and Rand Krochta, J.M. (1993), Dispersed phase particle size effects on water vapor Permeability of whey protein-beeswax edible emulsion film,. Journal Food Process Press, (18), pp. 173-188.

Moreno, O., Cardenas, J., Atarés, L., and Chiralt, A., (2017), Influence of starch oxidation on the functionality of starch-gelatin based active films, Carbohydrate Polymers, 178, pp. 147–158.

Pelissari, F.M., M.M. Andrade Mahecha, M.M., Sobral, P.J.d., and Menegalli. F.C., (2013), Comparative study on the properties of flour and starch films of plantain bananas (Musa paradisiaca), Food Hydrocolloids, 30(2), pp. 681-690.

Phan, T.D., Debeaufort, F., Luu, D., and Voilley, A., (2005), Functional properties of edible agar based and starch-based films for food quality preservation, Journal Agriculture Food Chemistry, (53), pp. 973–981.

Pietrzyk, S., Fortuna, T., Juszczak, L., Gałkowski, D., Małgorzat, B., Aczkowicz, Łabanowska, M., and Kurdziel, M., (2018), Influence of amylose content and oxidation level of potato starch on acetylation, granule structure and radicals’ formation, International Journal of Biology Macromolecule, 106, pp. 57–67.

Skurty, O., Acevedo, C., Pedreschi, F., Enrions, J., Osorio, F., and Aquilera, J.M., (2011), Food hydrocolloid edible films and coating. Department of Food Science and Technology. Chile: Universidad de Santiago de Chile.

Souza, A. C., Benze, R., Ferrão, E. S., Dichfield, C., Coelho, A. C. V., and Tadini, C. C. (2012), Cassava starch biodegradable films: Influence of glycerol and clay nanoparticles content on tensile and barrier properties and glass transition temperature, LWT Food Science and Technology, 46(1), pp. 110–117.

Thakur, R., Saberi, B., Pristijono, P., Golding, J., Stathopoulos, C., Scarletta, C., Bowyera, M., Vuonga, Q. (2016). Characterization of rice starch- l-carrageenan biodegradable edible film. Effect of stearic acid on the film properties. International Journal of Biological Macromolecules 93 (2016) 952–960.

Vásconez, M.B., Flores, S.K., Carmen A. Campos, C.A., Alvarado, J., and Gerschenson, L.N., (2009), Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings, Food Research International, 42, pp. 762–769.

Zhang, Y., and Han, J. H. (2006), Plasticization of pea starch films with monosaccharides and polyols, Journal of Food Science, 71(6), pp. 253–261.