

# Incorporation of Sodium Hyaluronate and Nyamplung

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## INCORPORATION OF SODIUM HYALURONATE AND NYAMPLUNG (*Calophyllum inophyllum*) CAKE EXTRACT TO IMPROVE BIOPLASTIC CHARACTERISTIC

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### ABSTRACT

The cross-linking agent plays an important role in bioplastic mechanical properties. This study aimed to determine the effect of Sodium Hyaluronate (SoHA) as a synthetic cross-linking agent and the addition of Nyamplung Cake Extract (NCE) as an antimicrobial agent in the manufacture of bioplastic with hydroxypropyl starch (HPS) as based ingredient using the thermo-compression method. The novelty of the study was thiocyanate (SCN) formation in zone 3 (2,161.66-2,162.02/cm) and cyanate (C-N=O) in zone 6 (1,411.57-1,412.61/cm) of (1, 2 and 3%) SoHA bioplastic and cyanate formation in zone 6 and 7 (1,411.37-1,558.59/cm) of (1, 2 and 3%) SoHA-20% NCE combined bioplastic originating from acetanilide group in SoHA and amide group in NCE. The formation of SCN and C-N=O in 2 and 3% SoHA bioplastic improved its sensitivity against gram-positive bacteria (*Staphylococcus aureus*) indicated by 0.6 mm and 0.45 mm inhibition zone, respectively. C-N=O formed in (1, 2 and 3%) SoHA-20% NCE combined with bioplastic had 3.25 mm average inhibition zone against gram-positive bacteria (*S. aureus*), 2.75 mm against gram-negative bacteria (*Escherichia coli*), and 0.71 mm against fungi (*Aspergillus niger*). Tensile analysis of mechanical properties showed that an addition of 3% SoHA was able to increase tensile strength and modulus of elasticity while reducing elongation, water solubility and water vapor permeability. Addition of (1, 2 and 3%) SoHA-20% NCE resulted in a reverse effect.

**Keywords:** acetanilide, amide, cross-linking agent, cyanate, thiocyanate

### INTRODUCTION

Natural polymers, such as starch, have various potential applications to replace commercial market-dominant petroleum-based plastics due to their abundant availability, degradability, renewability and low price (Samsudin & Hani 2019). The most critical property of plastic is its ability to be adjusted for specific purposes. The increasing utilization of starch for commercial use, such as to produce biodegradable thermoplastic, is expected to reduce problems caused by the petroleum-based polymer in solid waste disposal (Manoi & Rizvi 2010). As a renewable polymer source with film-forming ability, starch fulfills several

requirements as an alternative for plastic, such as having abundant availability, high extraction yield, cheap, biodegradable and biocompatible. Starch-based bioplastic is also odorless, tasteless, colorless, non-toxic, and semi-permeable to carbon dioxide, temperature, oxygen, as well as fat and aroma components (Shah *et al.* 2016).

Starch-based bioplastic as an environmentally friendly package has various disadvantages related to the hydrophilic and hygroscopic properties of starch. In this study, hydroxypropyl starch (HPS) as a modified starch was used as the main material, because HPS is more feasible to be processed into bioplastic due to its lower solubility temperature, with more transparent and flexible plastic properties (Woggum *et al.* 2015). Therefore, the mechanical properties of bioplastic can be improved and

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adjusted by adding a plasticizer <sup>6</sup> cross-linking agent. A plasticizer is a group of small molecules that are able to blend and interact among polymer <sup>6</sup>ains by intermolecular force, thus reduces glass transition temperature of a material to improve malleability, flexibility, extension and to soften the texture (Wypych 2017). On the other hand, cross-linking mechanism works by forming bridges of immediate <sup>4</sup>ermolecular bond by chemicals known as cross-linking agent. Cross-linking means polymer molecules are interconnected by bond (Canisag 2015). Cross-linking agent is substances that are able to form permanent chemical bond among polymer chains, <sup>6</sup>o increase material rigidity, firmness, strength, less flexible and increase glass transition temperature (T<sub>g</sub>). Cross-linking agent might affect permeability property, thermal stability, glass transition temperature and the rate of wear (Frost *et al.* 2013) due to formation of covalent or ionic bond connecting a polymer chain to the others which enhance polymer strength.

Cross-linking process is critical to modify starch properties by enhancing the starch's intra- and inter-molecular bonds in random patterns. The bonds tend to inhibit starch interaction to water while bringing structural integrity on starch-based biodegradable materials during hydraulic pressure and high humidity. Cross-linking process can be done by starch treatment, both in semi-solid or pulp form, using reagent with ability to form ether or ester bond among hydroxyl (-OH) in starch molecule (Manoi & Rizvi 2010). Cross-linking agents are able to form cross bond that enhances matrix density and tensile strength, and even permanent chemical bond among polymer chains, thus generate more rigid, strong, less flexible material and increase glass transition temperature (T<sub>g</sub>).

Polyfunctional chemicals such as phosphor oxychloride (POCl<sub>3</sub>), sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrine (EPI), a mixture of adipic, acetate anhydride, and mixture of succinic anhydride and vinyl acetate are some of the common cross-linking agents for starch. In this study, we used sodium hyaluronate (SoHA) as a cross-linking agent. SoHA is a polymer of disaccharides, consisting of D-glucuronic acid and D-N-acetylglucosamine, linked by β-1, 4 and β-1, 3 bonded by glycosidic bonds, into polymer

compounds that do not have UV absorbing chromophores (Ruckmani *et al.* 2013).

Hyaluronate supports the structure of connective tissue by acting as a magnet to maintain fluidity and form viscous liquids with lubrication properties. SoHA has low toxicity and no report of negative effect on human (Spec-Chem Ind. 2014). SoHA is also suitable as cross-linking agent and encapsulates various active ingredients (Corro 2018).

This study used nyamplung cake extract (NCE) as antimicrobial agent because of the ability of NCE against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*). Bioplastic is more sensitive against gram-positive bacteria compared to gram-negative bacteria. Bioplastic has the largest inhibition zone of 30 mm against *Staphylococcus aureus* and that of 23 mm against *Escherichia coli*. The ability of bioplastic as antimicrobial agent might be related to the content of the bioplastic extract which serves as natural cross-linking and antimicrobial agents (Umiyati *et al.* 2010).

The purpose of this study was to determine the effect of SoHA as synthetic cross-linking agent and nyamplung cake extract (NCE) as antimicrobial agent in the manufacture of bioplastic with HPS as basic ingredient using thermo-compression method. This study demonstrated that the features of bioplastic produced showed better mechanical properties, having antimicrobial ability compared to the conventional bioplastic.

## MATERIALS AND METHODS

### Materials

Nyamplung cake (*Calophyllum inophyllum* L.) was obtained from biodiesel industry in Purworejo, Central Java, Indonesia. Nyamplung cakes were sorted and dried until reaching moisture content below 10-15%. The dried cakes were finely ground and sifted using a 120-mesh sieve to obtain nyamplung cake powder. Subsequently, 20 g of the nyamplung cake powder was extracted by using 120 mL of 96% ethanol and placed on a hot plate stirrer at 80 °C for 1 h. The mixture was filtered using Whatman filter paper No. 1. Based on the optimal method

by Chana-Thaworn *et al.* (2011), the extracted liquid was then evaporated and oven-dried at 50 °C for 24 h. Other materials used in this study were hydroxypropyl starch (HPS), sodium hyaluronate (SoHA), glycerol 20%, ethanol 96% and distilled water.

**Instruments**

Thermo-compression machine, FTIR (Thermo Scientific Nicolet iS10), Scanning Electron Microscopy (JEOL JSM 6510) and texture analyzer (Brookfield USA) were used in this study.

**Bioplastic Preparation**

Various concentrations of SoHA (1%, 2% and 3% b/b) were mixed with 10 g of HPS, 2 g of nyamplung cake extract, 1.5 g of glycerol and 1 g of distilled water, then mixed at 21,000 rpm for 45 min until homogen. The mixture was then made into bioplastic using thermo-compression mold. The mixture was then placed in the middle of a 0.5 mm-thick aluminum frame sized 10 × 10 cm that was put between the two previously heated aluminum plates, molded at 140 °C and 250 kg/m<sup>2</sup> pressure for 6 min in heated hydrolic pressure machine (Rasheed *et al.* 2015).

Fourier Transform Infrared Spectroscopy (FTIRs) analysis is a method to identify structural changes in starch chains due to interaction among extract, glycerol, NaHA, and HPS molecules (Bilal *et al.* 2015). FTIR spectra of bioplastic was measured by Thermo Scientific Nicolet iS10, equipped with Smart ATR Diamond at an area of 650-4,000/cm using 32 scanning and resolution of 8/cm.

Scanning Electron Microscopy (SEM) was used to analyze microplastic structure of the bioplastic. Sample of bioplastic was stored in a desiccator with P<sub>2</sub>O<sub>5</sub> absorbent for two weeks to ensure no moisture left in the bioplastic sample. For cross-section observation, the bioplastic sample was frozen in N<sub>2</sub> liquid for cryofracture preservation (Espinel *et al.* 2014). All samples were placed in bronze stub and covered with gold sheets before imaging. Micrograph of the surface of bioplastic and fracture was obtained using SEM (JSM-6510LA) at 10-300,000x magnification and 1-10 nm resolution.

**Characterization of Bioplastic Physical Properties**

Mechanical properties such as tensile strength (σ), elongation (ε), modulus of elasticity (Y), water solubility (WS) and water vapor permeability (WVP) were measured based on the methods in ASTM D638. Prior to mechanical properties analysis, the bioplastic sample was conditioned at RH of 50 ± 5 and 23 °C for 48 h in humid chamber. Tensile strength at room temperature was measured using Brookfield USA analyzer texture. Bioplastic specimen was cut according to the method by ASTM D1708 at a constant pull rate of 5 mm/minute. Solubility measurement of bioplastic sample in water was conducted by drying the bioplastic sample at 105 °C and then weighed as m<sub>3</sub>. Subsequently, the bioplastic sample was soaked in 50 mL distilled water at 24 °C for 6 h, then oven-dried at 105 °C for 24 h and weighed as m<sub>4</sub> (Hassannia-kolace *et al.* 2016; Kumari *et al.* 2017), before calculation using formula below (1):

$$WS = \frac{m_3 - m_4}{m_4} \times 100 \dots\dots\dots (1)$$

where: m<sub>3</sub> = weight of bioplastic sample before drying  
 m<sub>4</sub> = weight of bioplastic sample after drying

Water vapor permeability (WVP) was analyzed using standard method in ASTM (1996) E96. The bioplastic sample was placed as a cover on circle permeation cells sized 4 cm in diameter containing silica gel (0% RH) and placed in a desiccator which was previously filled with saturated sodium chloride liquid (75% RH), then stored at 30 °C. Water vapor permeability rate was measured by permeation cell weighing at a frequency of every 30 min to obtain several points. The WVP was counted using a formula (2) developed by Wirawan *et al.* (2012):

$$WVP = \frac{WPTR}{P_s (RH_1 - RH_2)} \times \delta m \dots\dots\dots (2)$$

where:  
 WVP = water vapor permeability (g.mm/KPa.s.m<sup>2</sup>)  
 WVTR = mass increase (g)  
 P<sub>s</sub> = pressure of saturated water vapor (Pa)

$RH_1$  = relative humidity in desiccator  
 $RH_2$  = relative humidity in permeation cell  
 $\delta m$  = average thickness of bioplastic layer (m)

### Characterization of Bioplastic Antimicrobial Properties

Disk agar method was used for analyzing the antimicrobial activity of bioplastic sample using distilled water as solvent. As much as 0.1 mL of 10% bacteria and fungi suspension was inoculated on Mueller Hinton Agar and on Potato Dextrose Agar media, respectively. Bioplastic sample of 1 cm diameter was placed on a sterile disk sized 6 mm diameter and let to dry. Subsequently, the bioplastic sample was placed on the surface of culture media in a Petri dish that was previously inoculated with tested microorganisms, then incubated at 37 °C for 1-2 days for bacteria and at 30 °C for 2-3 days for fungi, based on optimum condition reported by Chanwitheesuk *et al.* (2007).

## RESULTS AND DISCUSSION

### Bioplastic Characteristics

#### Mechanical Properties

Comparison of mechanical properties in the form of tensile strength ( $\sigma$ ), elongation at break ( $\epsilon$ ) and modulus of elasticity (Y) of bioplastic

prepared with (1, 2 and 3%) SoHA, combination of (1, 2 and 3%) SoHA-20% NCE compared to that prepared by using 20% NCE and control is presented in Figure 1. Those mechanical parameters depend on microstructure characteristics (Aguirre *et al.* 2013).

Bioplastic thickness ranged from 0.24 to 0.32 mm (data not shown). Results of this study showed that the control bioplastic had significant increase of tensile strength ( $\sigma$ ) compared to 3% SoHA bioplastic and 20% NCE bioplastic, by 23.7% and 50.8%, respectively, while that of (1, 2 and 3%) SoHA-20% NCE bioplastic had significant reduction by an average of 62.4% (Fig 1). In terms of elongation at break ( $\epsilon$ ), the (2 and 3%) SoHA bioplastic showed significant reductions compared to the control bioplastic by an average of 32.4%, while the (1, 2 and 3%) SoHA-20% NCE bioplastic had a significant increase of 224.2%. For modulus of elasticity (Y), there was significant difference between the control and (1, 2 and 3%) SoHA, (1, 2 and 3%) SoHA-20% NCE combination, and 20% NCE. This indicated that SoHA addition to 3% could function as effective cross-linking agent to improve bioplastic mechanical properties. SoHA works to facilitate esterification of starch OH groups in overcoming the poor polymer compatibility by incorporating compatible substances (Ortega-toro *et al.* 2014).

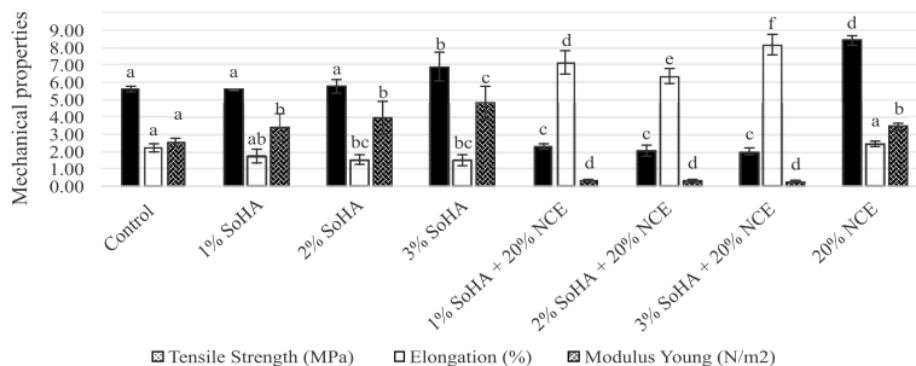


Figure 1 Effect of SoHA addition and combination of SoHA-NCE on the mechanical properties of HPS bio-plastic  
 Notes: Bar indicates mean  $\pm$  standard deviation. Different letters in the same column indicate significant differences ( $P < 0.05$ ).

SoHA is a widely distributed natural mucopolysaccharide polymer, which in mammals is known as connective tissue. Cross-linking process is done by SoHA to increase the *in vivo* retention time, so that the hydroxyl groups within the polymer chains bind to one another. The cross-linking reaction in SoHA is carried out in a homogeneous aqueous alkaline solution which can enhance anti-biodegradation ability by increasing the degree of cross-linking and extending the *in vivo* retention time (Xuejun *et al.* 2015).

Utilization of the modified HPS starch as basic material may also serve to improve bioplastic mechanical properties due to its high amylose content of modified starch. Therefore, HPS is feasible as bioplastic raw material, has mechanical strength and functions as good oxygen inhibitors (Woggum *et al.* 2015). Concentration of cross-linking agent, pH, treatment temperature and storage period might determine cross-linking bond. Reaction condition can be varied according to the type of cross-linking agent (Canisag 2015).

Utilization of SoHA-NCE combination reduced  $\sigma$  and  $Y$ . Higher concentration of SoHA on NCE resulted in a lower  $\sigma$ . These effects were probably due to the loss of amide group in NCE bioplastic (20% extract) by the combination. On the other hand, SoHA-NCE combination increased  $\epsilon$ , which probably due to  $H_2O$  formation from reactions involving SoHA and hydroxyl groups from HPS starch, glycerol-SoHA amide groups of NCE-SoHA.  $C-N=O$  is an intermediate product of the first stage of thiocyanate hydrolysis, which was then hydrolyzed into ammonia and bicarbonate (Doble & Kumar 2005).

Stronger interaction of HPS-SoHA-glycerol reduces  $Y$  and maximum tension as well as tension-when-separate resulted in a more flexible bioplastic, and therefore, showing the function of SoHA as cross-linking agent (Contipro 2018). Modified starch was reportedly a more stable raw material for making bioplastic compared to native starch (Gutiérrez *et al.* 2015). Bioplastic preparation might also play a role as thermo-compression utilizes high temperature and hydraulic pressure. There was also the possibility of maillard reaction which can form more compact networks (Leceta *et al.* 2013).

HPS as raw material might affect bioplastic mechanical properties due to the effect of hydroxypropylation that interfere the inter- and intra-molecular hydrogen bond of the starch chain. The effect can enervate starch granule while increasing starch chain movement in an amorph area and enhancing HPS swelling power compared to native starch with increasing molar substitution (Woggum *et al.* 2015). SoHA addition brought significant increase on  $\sigma$  at  $\geq 3\%$ , but NCE addition in SoHA bioplastic did otherwise.

### Water Solubility (WS)

SoHA and SoHA-NCE combination affected bioplastic water solubility (WS) in terms of bioplastic integrity in watery media. High solubility of bioplastic indicate low water resistency (Gutiérrez *et al.* 2015). WS is defined as the ratio of dry matter in water, such as bioplastic soaked in distilled water (Hassannikolae *et al.* 2016). Results of this study showed that WS of bioplastic made by using SoHA, combination of SoHA-NCE and NCE were significantly lower than that of control (Fig. 2).

SoHA bioplastic when compared to control had an average of lower WS values by 6.6%, while bioplastic made by using SoHA-NCE combination had an average of lower values by 15.9%, then bioplastic made by using NCE had an average of lower values by 20.95%. Insignificant WS decrease was observed in 2% SoHA compared to 3% SoHA bioplastics and vice versa, as well as in 1% SoHA-20% NCE combination compared to NCE bioplastics (Fig. 2). This showed that NCE addition affected the molecular structure of SoHA bio-plastic.

During bioplastic preparation, phosphor of STMP (as a cross-linking agent in making modified starch) reacted with hydroxyl (-OH) group of starch to form phosphate distarch (cross-bond) and other phosphate derivatives as indicated by the FT-IR results. Cross-linking process enhances granule structure starch and inhibit both water absorption and starch solubility, thus limit the mobility of starch chain in amorph area (Manoi & Rizvi 2010). Bioplastic prepared by HPS-SoHA had lower solubility compared to control, with increasing concentration of cross-linking agent. This

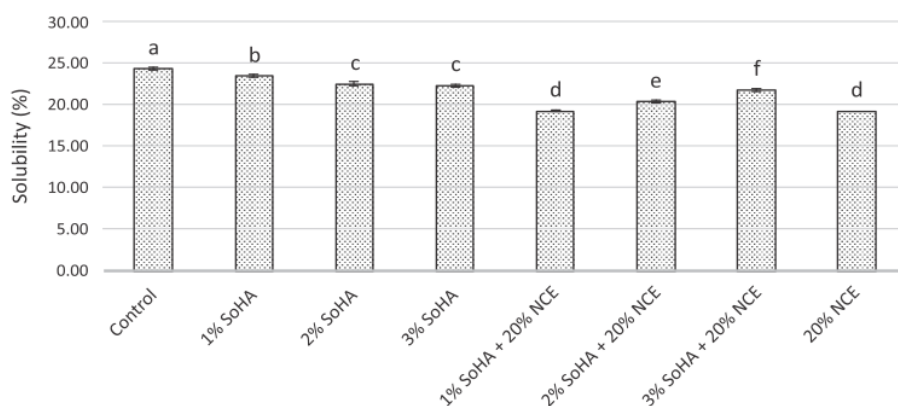


Figure 2 Effect of SoHA addition and combination of SoHA-NCE on the water solubility of HPS bio-plastic

Notes: Bar indicates mean  $\pm$  standard deviation. Different letters in the same column indicate significant differences ( $P < 0.05$ ).

finding was comparable to previous study conducted by (Manoi & Rizvi 2010) which indicated that the formation of phosphate distarch act as cross-linking agent might limit swelling and hydration of starch granules. Hyaluronate is known as a hydrophilic polymer derivative polysaccharide that has ability as an enhancer of percutaneous penetration by changing composition of cells of tightly arranged materials into more tenuous so that permeability is increased. Permeability has an inverse relationship with WS so that the addition of SoHA can decrease WS of bioplastics (Djajadisastra *et al.* 2014). WS is an important property of bioplastic for food prevention application, particularly in high water activity, or when bioplastic must be in contact with water, such as during food processing. Generally, high solubility indicates lower water endurance, though high solubility might be advantageous for some applications (Chana-Thaworn *et al.* 2011). According to (Spec-Chem Ind. 2014), SoHA can function as a lubricant and film maker, where SoHA is a high molecular weight polymer which is a strong lubricant and film maker.

SoHA as cross-linking agent can be observed from its ability to reduce the WS of bioplastic. It

was contrary to the results obtained from SoHA-NCE that increased WS due to water formation from reaction between HPS, SoHA and NCE, showing the hydrophilic property of bioplastic.

#### Water Vapor Permeability (WVP)

WVP is a bioplastic ability to withhold penetrating water vapor and provides information on water vapor transmission through bioplastic as a critical property of food packaging (Leceta *et al.* 2013). Bioplastic permeability is determined by the difference of water vapor concentration between one-side to the other side of bioplastic, with higher difference indicating faster mass transfer and also affected by bioplastic thickness. The results of WVP analysis showed that the control bioplastic had significant difference compared to bioplastic prepared by using 3% SoHA, SoHA-NCE combination and NCE. Increasing concentration of 3% SoHA and 20% NCE bioplastic was able to decrease WVP by an average of 3.66% and 3.89%, respectively, while (1, 2 and 3%) SoHA-20% NCE combination on the contrary increased WVP by an average of 8.25% (Fig. 3).

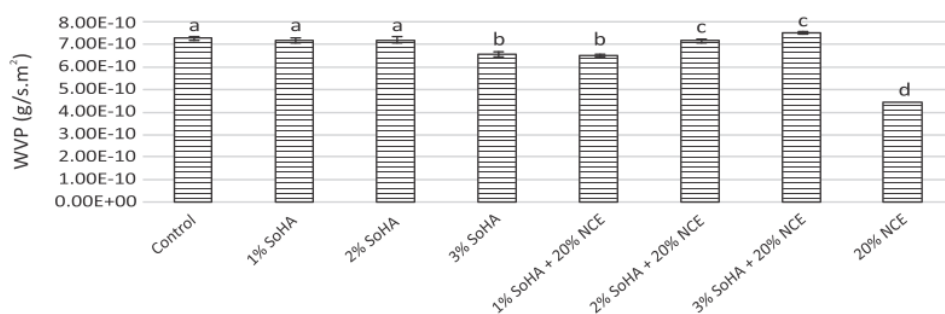


Figure 3 Effect of SoHA addition and combination of SoHA-NCE on the WVP of HPS bioplastic  
Notes: Bar indicates mean  $\pm$  standard deviation. Different letters in the same column indicate significant differences ( $P < 0.05$ ).

The results of WVP analysis showed that the control bioplastic had significant difference compared to bioplastic prepared by using 3% SoHA, SoHA-NCE combination and NCE. Increasing concentration of 3% SoHA and 20% NCE bioplastic was able to decrease WVP by an average of 3.66% and 3.89%, respectively, while (1, 2 and 3%) SoHA-20% NCE combination on the contrary increased WVP by an average of 8.25%. Previous study conducted by Gutiérrez *et al.* (2015) reported a tendency of increasing WVP and WS in bioplastic containing native starch, which indicated higher rate of hydrophilic property. Another possibility for increasing WVP in (1, 2 and 3%) SoHA-20% NCE bioplastic was by implementing higher hydrophilic property in the (1, 2 and 3%) SoHA-20% NCE bioplastic compared to (1, 2 and 3%) SoHA and 20% NCE bioplastic.

Water holding capacity of SoHA is very high compared to other moisturizers. The constant of SoHA moisture evaporation rate is lower than that of other moisturizers. This shows that SoHA has strong water retention properties, with moisture evaporation rate constant of  $8.0 \times 10^{-2}/\text{min}$  (Spec-Chem Ind. 2014). The main function of bioplastic or edible film is to inhibit vapor transfer from the surrounding environment to the food covered by the bioplastic, or between two different components of food products. Therefore, WVP value needs to be as low as possible (Chana-Thaworn *et al.* 2011). WVP is also determined by bioplastic thickness as well as by the glycerol and starch concentrations. Thickness of hydrophilic film might determine WVP, with higher thickness resulted in higher resistance against mass

transfer and increasing partial water pressure in equilibrium of film inner surface, thus enhance water vapor permeability of film hydrophilic property with higher thickness (Gutiérrez *et al.* 2015). The enhancing WVP is caused by the changes of partial water vapor pressure of the exposed inner surface of the film.

Separate utilization of SoHA and 20% NCE was able to reduce WVP, indicating their function as cross-linking agent due to their ability to inhibit water vapor penetrating the bioplastic. On the other hand, different results occurred in bioplastic made from a combination of (1, 2 and 3%) SoHA-20% NCE due to deteriorated cross-linked bond from water formation which subsequently caused bioplastic became hydrophilic.

### 35 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The recent development of material technique has resulted in demand increase for fast, reliable and non-destructive analytical methods to control preparation process and physicochemical characterization. FTIR provides general information on chemistry of the surface and overall characters as well as their functions in polymer (Ricci *et al.* 2015). FTIR spectra of control, (1, 2 and 3%) SoHA, (1, 2 and 3%) SoHA-20% NCE and 20% NCE bioplastic was presented to measure the results of reaction involved in bioplastic preparation made from HPS with SoHA as cross-linking agent, as well as combination of SoHA-NCE (Fig. 4).



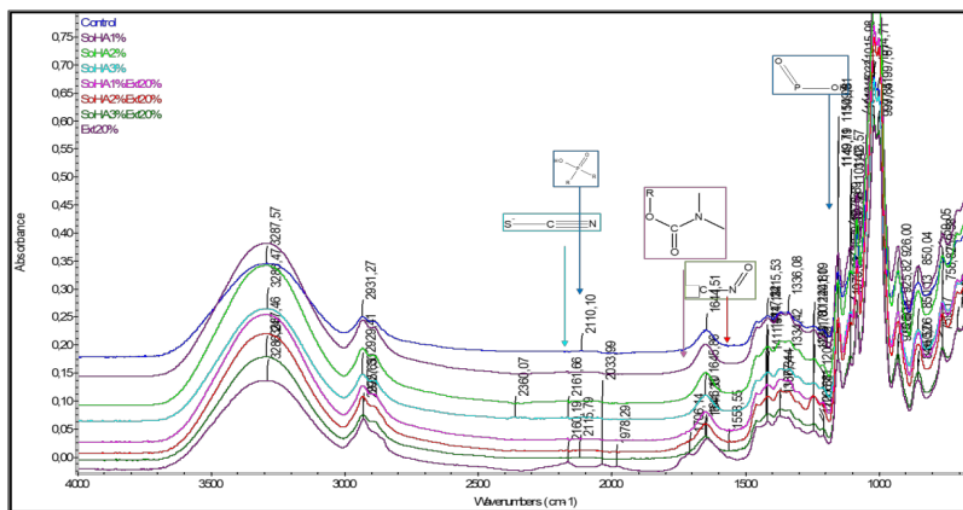


Figure 4 FTIR Spectra of control, (1, 2 and 3%) SoHA, (1, 2 and 3%) SoHA-20% NCE and 20% NCE bioplastics

Differences in several peaks of bioplastic made by using 20% NCE compared to (1, 2, and 3%) SoHA and combination of (1, 2 and 3%) SoHA-20% NCE (Fig 4; Table 1). Zone 1 representing bioplastic made by using SoHA at 3,285.91-3,288.09/cm; SoHA-NCE at 3,287.46-3,297.68/cm; and NCE at 3,282.13/cm showed OH bond vibration, related to bound, free, inter- and intra-molecular hydroxyl group (Bilal *et al.* 2015). Zone 2 representing bioplastic made by using SoHA at 2,926.41-2,927.94/cm; SoHA-NCE at 2,927.28-2,927.61/cm; and control at 2,924.49/cm indicated stretching vibration of ketones (CH<sub>3</sub>-CO). Zone 3 representing bioplastic made by using SoHA at 2,161.66-2,162.02/cm and 1% SoHA-20% NCE showed stretching vibration of SCN (isocyanates). SCN can be derived from cyanide-rich plants, such as cassava, sweet potato, corn, sugar cane, sorghum and linseed (Chandler & Day 2012).




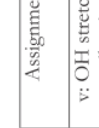
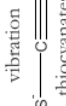
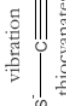

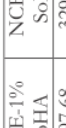


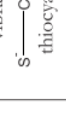


SCN presence in (1, 2, and 3%) SoHA bioplastic was probably derived from corn starch rich as HPS raw material. SCN functions as host defense and as cyanide detoxification product. SCN is a preferable substrate for lipid peroxidation (LPO), for catalytic reduction caused by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into hypocyanate acid (HOSCN) (Chandler & Day 2012). Eighty percent (80%) SCN in the body comes from cyanide absorbed from food which transformed into SCN in metabolism (Simeonova & Fishbein 2004). In addition, the

presence of SCN vibrations may originate from reaction between acetanilide group in SoHA and sulfate compounds from HPS.

Zone 4 representing (2% and 3%) SoHA and (1, 2, and 3%) SoHA-20% NCE bioplastic showed OH-P=O bond area, which arguably indicated cross-linking activity through transformation of starch granule by bifunctional or multifunctional reagents which were able to form ether or ester bond with hydroxyl group of starch (Gui-jie *et al.* 2006). In zone 8 (1,411.37-1,558.59/cm) of (1, 2 and 3%) SoHA-20% NCE bioplastic and zone 9 (1,411.57- 1,412.61/cm) of (1, 2 and 3%) SoHA bioplastic, cyanate (C-N=O) was formed. Whereas, in the 20% NCE bioplastic at 1,704.17/cm, amide group was present. This indicated that NCE combined with (2 and 3%) SoHA removed SCN vibration. Besides hydroxyl group and glycerol, amide group from NCE and acetanilide from SoHA also played important roles in determining bioplastic mechanical properties as well as cyanate (C-N=O) formation observed in peak (1,558.46-1,558.59) (Fig. 4; Table 1).

There was a higher presence of C-N=O in (1, 2 and 3%) SoHA-20% NCE compared to that in (1, 2 and 3%) SoHA bioplastic which probably caused by NCE addition, in dose-dependent manner with the decreasing cyanide concentration due to oxidation. Cyanide can be photocatalytic oxidized into C-N=O,

Table 1 Assignment FTIR Spectra of (1, 2 and 3%) SoHA, (1, 2 and 3%) SoHA-20% NCE and 20% NCE bioplastics

Peaks	SoHA1%	SoHA2%	SoHA3%	Assignment	NCE-1% SoHA	NCE-2% SoHA	NCE-3% SoHA	Assignment	Ext20%	Assignment
1	3285.91	3288.09	3286.47	v: OH stretching vibration Cellulose	3297.68	3296.74	3287.46	v: OH stretching vibration Cellulose	3299.94	v: OH str. Vib 
2	2927.95	2929.50	2926.41	w: CH <sub>3</sub>	2927.28	2927.61	2927.30	w: CH <sub>3</sub>	2924.49	w: CH <sub>3</sub>
3	2162.02	2161.91	2161.66	s-m: CN stretching vibration 	2162.29			s-m: CN stretching vibration 	2159.83	s: aromatic selenocyanates 
4		2114.74	2114.90	w-m: broad 	2115.05	2115.65	2115.79	w-m: broad 	2159.83	w-m: broad 
5									2034.31	v: broad, asym. CNN stretching vibration 
6									1978.33	Diazo compounds Aromatic system 
7									1704.17	s: 
8					1558.59		1558.55	s: monomer 		
9	1412.61	1412.76	1411.57	s: 	1417.19	1411.37	1417.22	s: 	1414.46	s: RO-SO <sub>2</sub> -OR cyanate

particularly in alkali media (Destanoğlu & Gümüş-Yılmaz 2016). The photocatalytic oxidation was shown by increasing the rate of C-N=O formed in (1, 2 and 3%) SoHA-20% NCE bioplastic due to NCE addition, which low pH was caused by hydrolyzed tannin dominated by gallic acid. In zone 10, 13 and 14, both SoHA and SoHA-NCE bioplastics, ether group was formed as an indication of cross-linking process between hydroxyl group of HPS and gallic acid or amide from hydrolyzed tannin. Moreover, peaks dominated by ether and aromatic groups also found at peaks 15, 16, 18 and 19. Utilization of (1, 2 and 3%) SoHA in HPS bioplastic preparation caused SCN and C-N=O formation, while 20% NCE addition caused increasing C-N=O concentration that arguably determined bioplastic mechanical properties and antimicrobia activity.

### Scanning Electron Microscopy Analysis

Micrograph resulted from the Scanning Electron Microscopy (SEM) analysis was used to study the tensile surface fracture of the eight bioplastic samples and microstructure changes caused by the addition of (1, 2 and 3%) SoHA and combination (1, 2 and 3%) SoHA-20% NCE.

The control bioplastic had homogenous surface microstructure with cracks in several parts. The microcracks formation was possibly

caused by absence of cross-linking agent in bioplastic (Ortega-toro *et al.* 2014). Addition of cross-linking agent can be detected by white spots of agglomeration on bioplastic surface made by applying addition of (2 and 3%) SoHA. The spots appeared as a side effect of uneven mixing or lack of solvent to dissolve cross-linking agent. Different to bioplastic microstructure made by using (1, 2 and 3%) SoHA, those made by using (1, 2 and 3%) SoHA-20% NCE combination had a very significantly different result compared to control, (1, 2 and 3%) SoHA and 20% NCE. Addition of 20% NCE resulted in aggregated formation on bioplastic surface, hence more flexible compared to other bioplastic samples. The flexibility was as also indicated by higher  $\epsilon$  of bioplastic made by using (1, 2 and 3%) SoHA-20% NCE compared to the other bioplastic samples (Fig. 5).

SEM micrograph of the eight bioplastic samples showed significantly different results, with (2 and 3%) SoHA bioplastic had agglomeration as indication of cross-linking agent addition, whereas (1, 2 and 3%) SoHA bioplastic had more flexible texture from water formation because the final reaction product rendered hydrophilic property of bioplastic. NCE contains organic acid having plasticizing effect that contributes to inhibiting starch partial recrystallization (Ortega-toro *et al.* 2014).

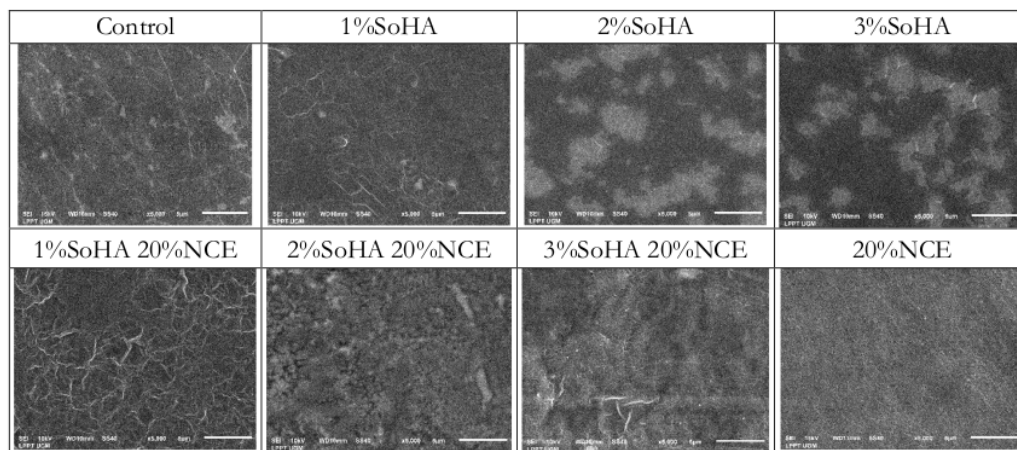


Figure 5 SEM micrographs of the control, (1, 2, and 3%) SoHA, (1, 2, and 3%) SoHA-20% NCE and 20% NCE bioplastics

### Antimicrobial Analysis

Antimicrobial capabilities from control bioplastic was compared to that of (1, 2 and 3%) SoHA, (1, 2 and 3%) SoHA-20% NCE and 20% NCE bioplastic<sup>3</sup> by determining the inhibition zone diameter against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria, as well as fungi (*A. niger*).

The results showed that control and 1% SoHA had no antimicrobial activity, although the FTIR spectrum analysis showed that there is a cyanate suspected as an antimicrobial agent. The lack of antimicrobial activity was probably due to the small concentration of the cyanate. The 2 and 3% SoHA had inhibition zone indicating antibacteria activity against gram-positive bacteria (*S. aureus*) with diameter of 0.6 mm and 0.45 mm, respectively (Table 2).

The presence of antimicrobial activity was probably caused by the presence of SCN and C-N=O in the SoHA addition during bioplastic preparation, or in the SoHA addition within the HPS which generated SCN and C-N=O groups. SCN can be generated from catalysis of cyanide (Fawell *et al.* 2007). Other study conducted by (Destanoğlu & Gümüş-Yılmaz 2016) mentioned that SCN is a chronic exposure of low dose of cyanide. Besides, SCN<sup>20</sup> is utilized in various industrial process, such as photofinishing, production of herbicide and insecticide, colorant, production of acrylic fiber, thio-urea production, metal separation and coating, as well as soil sterilization and corrosion inhibition (Doble & Kumar 2005). NCE addition to (1, 2 and 3%) SoHA-20% NCE bio-plastic, w<sup>3</sup> able to improve antimicrobial properties against gram-positive (*S. aureus*) and gram-negative

(*E. coli*) bacteria as well as fungi (*A. niger*). Antimicrobial activity of (1, 2 and 3%) SoHA-20% NCE bioplastic was considered better than the control, (1, 2 and 3%) SoHA and 20% NCE, which probably due to the presence of C-N=O that was predictably increased with the increasing NCE. The existence of C-N=O is probably derived from acetanilide group found in SoHA and amide group contained in NCE. Acetanilide is the first derivative of aniline having analgesic and antipyretic properties (Khan *et al.* 2016). C-N=O concentration can be gradually increased with time, in line with the reduction of cyanide due to oxidation (Destanoğlu & Gümüş-Yılmaz 2016). The analysis results showed that (1, 2 and 3%) SoHA-20% NCE bioplastic was more sensitive against *S. aureus*<sup>19</sup> and *E. coli* or *A. niger* (Table 2). This indicated that *S. aureus* was more sensitive to C-N=O in (1, 2 and 3%) SoHA-20% NCE bioplastic. Different results were obtained by 20% NCE bioplastic containing amide, toward which *E. coli* had higher sensitivity than *S. aureus*, with no antimicrobial activity against *A. niger*.

Results of this analysis also indicated that gram-positive bacteria (*S. aureus*) were more sensitive toward SCN or C-N=O in both 3% SoHA and (1, 2 and 3%) SoHA-20% NCE bioplastics compared to gram-negative bacteria (*E. coli*) and fungi (*A. niger*). However, C-N=O compounds provided antimicrobial activity for (1, 2 and 3%) SoHA-20% NCE bioplastic against *S. aureus*, *E. coli* and *A. niger*. On the other hand, amide content in 20% NCE showed higher sensitivity against *E. coli* than *S. aureus*, with no antimicrobial activity against fungi (*A. niger*).

Table 2 Inhibition zone obtained in the antimicrobial analysis

No	Sample	Inhibition zone against		
		<i>E. coli</i>	<i>S. aureus</i>	<i>A. niger</i>
1	HPS-0	0a	0a	0a
2	HPS-0-SoHA-1	0a	0a	0a
3	HPS-0-SoHA-2	0a	0a	0a
4	HPS-0-SoHA-3	0a	0.45 ± 0.26b	0a
5	HPS-20-SoHA-1	3.17 ± 0.29b	3.57 ± 0.55c	0.77 ± 0.12b
6	HPS-20-SoHA-2	2.67 ± 0.06c	3.23 ± 0.31c	0.80 ± 0.00b
7	HPS-20-SoHA-3	2.40 ± 0.17d	2.97 ± 0.42d	0.57 ± 0.06c
8	HPS-20	5.07 ± 0.20e	4.32 ± 0.25e	0a

## CONCLUSION

Addition of (1, 2, and 3%) SoHA as cross-linking agent and combination of (1, 2, and 3%) SoHA-20% NCE had significant effects on mechanical properties and antimicrobial activity of HPS bioplastic. SoHA at 2 and 3% in HPS bioplastic improved mechanical properties in form of  $\sigma$  and Y increase while reducing  $\epsilon$ , WS and WVP. The reduction was caused by the presence of SCN and C-N=O which probably derived from an acetanilide group found in SoHA causing antimicrobial activity against gram-positive bacteria (*S. aureus*). On the contrary, combination of (1, 2 and 3%) SoHA-20% NCE degraded mechanical properties by reducing  $\sigma$  and Y followed by  $\epsilon$ , WS and WVP decrease, which probably due to water formation from reactions involving HPS, SoHA, and NCE. However, antimicrobial analysis indicated sensitivity against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria as well as fungi (*A. niger*) which arguably caused by C-N=O formation increase in bioplastic, originating from acetanilide group in SoHA and amide group in NCE.

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