



# **CHITOSAN AND LAURIC ACID ADDITION TO CORN STARCH-FILM BASED EFFECT: PHYSICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY STUDY**

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## **ABSTRACT**

Biodegradable starch film has potential applications in agriculture, food, and pharmacy. However, film made solely of starch possesses poor water resistance, mechanical properties, and antimicrobial activity. In this experiment, some films were prepared by combining starch, chitosan, and lauric acid in various ratios in order to improve those properties. The film thickness, moisture content, tensile strength, elongation, water vapour permeability (WVP), and melting point were investigated. The possible interactions among components were evaluated by Fourier-transform infrared spectroscopy (FTIR) and antimicrobial activity was examined by using agar diffusion method. The mixed film exhibited higher tensile strength and lower WVP than that of the starch film. The amino group band in the FTIR spectrum shifted from  $1563\text{ cm}^{-1}$  in pure chitosan film to  $1562\text{ cm}^{-1}$  in the mixed film. Antimicrobial assay showed that the mixed film owned a moderate inhibition response to Gram positive and low response to Gram negative bacteria. This film is not feasible yet to be marketed because its mechanical and antimicrobial properties are still below the standard.

**Key words:** Antimicrobial, Biodegradable, Chitosan, Mechanical properties, Starch.

## **INTRODUCTION**

Nowadays, around 150 million ton of plastics are produced every year in all around the world. This amount increases continuously whether from production or consumption side. Mostly, plastics are produced from petroleum derivative. It can make serious environmental pollution as impact from not degradable waste<sup>1</sup>. Using biopolymer of agricultural product, which is easy to be degraded is one of solutions. Not only overcome that problem, but also gives additional value to agricultural product<sup>2</sup>.

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Starch is natural polymer, which is very suitable for edible film raw material since it can be degraded by microorganism or is enzymatically degraded into glucose, which is degraded into carbon dioxide and water afterwards. Other than its biodegradable property, starch is also abundant and the production cost is also low<sup>3</sup>. Starch is really hydrophilic and since it is really hygroscopic, this film permeability to water is high enough, so it gives difficulty to avoid water vapor transmission at the formed film<sup>4</sup>. Some researches related with starch-based film conducted by Lopez et al.<sup>5</sup>, Maran et al.<sup>6</sup> and Woggum et al.<sup>7</sup> Lopez et al.<sup>5</sup> reported that combination of original starch with acetylated starch has potential to be biodegradable film. Adding acetylated starch is able to improve the heat property from original starch-based film. Maran et al.<sup>6</sup> reported that glycerol and emulsifier (span 80) addition is able to increase starch film flexibility, while Woggum et al.<sup>7</sup> reported that rice starch modification by 6-12% propylene oxide followed by cross linking with 2% sodium trimethaphosphate can decrease that water vapor transmission. Starch-based film improvement has taken a lot of efforts. Other than improve mechanical properties and film stability, modification is aimed to increase its function. The desired function from this film is to be an edible film having potential to inhibit bacterial or microbial growth.

Packaging material having broad antimicrobial spectrum is really desired to increase resilience store of various food stuffs. Because of that, adding antimicrobial material to packaging material is generally applied. Chitosan, de-acetylated polysaccharide product from chitin, is one of materials generally added to reach that aim since chitosan has antimicrobial property and is able to inhibit various fungi, yeast, and bacteria growth<sup>8</sup>. Chitosan addition has dual function, because other than its antimicrobial property, chitosan is also be able to improve mechanical properties from this starch-based film. Starch combined with chitosan gives increased tensile film<sup>9</sup>. Other than its antimicrobial property, this film is also desired to avoid water vapor transmission. Therefore, material having ability to suppress starch hydrophilic property is also required for this film. One of additive materials, which are able to overcome this weakness is fatty acid.

Fatty acid is an aliphatic compound having carboxyl groups. Based on its chain length, it can be divided into 3 categories. First, is short chain fatty acid (SCFA) having less than 8 carbon atom, then medium chain fatty acid (MCFA) with 8-12 carbon atoms, and long chain fatty acid (LCFA) having more than 12 carbon atoms. While based on the double bond existence, there are unsaturated fatty acid and saturated fatty acid. Lauric acid is belonging to saturated fatty acid with moderate chain length (12 carbon atoms). It gives impact to high hydrophobic property. Adding this fatty acid to starch-based film can decrease water vapor transmission<sup>10</sup>. Saleh and Muhamad<sup>11</sup> reported that lauric acid addition

to wheat starch-based film is able to decrease hydrophilic property and excess water vapor transmission from the formed film.

In this research, corn starch is used as starch source. This starch is composed of 25% amylose and 75% amylopectin while wheat starch is composed of 52% amylose and 48% amylopectin. Here, corn starch is combined with chitosan and also lauric acid to achieve edible film having antimicrobial ability, strong mechanical properties, and avoid water vapor transmission, which is not done yet. Therefore, this research is aimed to evaluate chitosan and lauric acid addition effect to antimicrobial ability, water vapor permeability, tensile, and elongation from corn starch-based biodegradable film. This antimicrobial ability is compared to *Escherichia coli* and *Bacillus subtilis*. Combining chitosan and lauric acid to starch-based film is desired to be synergy to increase antimicrobial ability and strengthen film mechanical properties, so this film has potential to be developed as edible antimicrobial package.

## EXPERIMENTAL

### Materials and methods

Materials used are corn starch (PT Gandum Mas Kencana), Chitosan with 70% de-acetylation degree (Brataco), lauric acid 98% (Merck), glycerol p.a (Merck), concentrated acetic acid (Merck), *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). Equipment used are micrometer, Fourier transformation infrared spectrophotometer (FTIR) Perkin Elmer type spectrum one, differential scanning calorimeter (DSC) model Netzsch DSC 200F3, tensile and elongation tester model SSB 0500.

### Starch film preparation<sup>11</sup>

Starch mixture (5.0 g), glycerol (2.5 g), and water (92.5 g), without lauric acid and chitosan is used as starch blank. While chitosan blank is made by dissolving 8 g chitosan into distilled acetic acid solution 5% (v/v) until 400 mL. Starch and chitosan solution are then mixed with some ratio [8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (b/b)], and then glycerol is added (half portion of starch weight) and also 8% lauric acid (lauric acid weight per starch weight). The mixture is then stirred with magnetic stirrer until starch is dissolved perfectly. After that, the solution is homogenized while heating slowly. Stirring and heating are stopped when the solution reach 70°C. As much as 5 mL film solution is moved by pipette and poured to petri dish and then air-dried in room temperature for a night. After that, film thickness is measured by micrometer for 5 times at the middle, right top, right bottom, left top, and left bottom corners, then average value is calculated.

## Starch-based film physical and chemical characterization

### Water content determination<sup>12</sup>

Porcelain dish is heated for 30 mins at 105°C and is weighed. As much as ± 0.1000 g of sample is weighed into that dish, and then is heated at 105°C for 3 hrs. After that, that dish containing sample is cooled down in dessicator for 30 mins and is weighed. This stage is repeated until constant weight is achieved. Water content is calculated by this formula –

$$\text{Water content} = \frac{A - B}{A} \times 100\%$$

Remark: A = Initial sample weight (g)

B = Sample weight after drying (g)

### Tensile and elongation analysis<sup>13</sup>

Film is cut by size 80 × 20 mm<sup>2</sup>. Piece of film is clamped by tensile tester and is pulled with constant rate. The tester will pull the film until break. Tensile value and elongation can be calculated by this formula:

$$\sigma = \frac{F}{A}$$

$$\% E = \frac{\Delta L}{L_1} \times 100\%$$

Remark:  $\sigma$  = Tensile (MPa)

$F$  = Pull force (N)

$A$  = Force area (mm<sup>2</sup>)

$\% E$  = % elongation (%)

$\Delta L$  = Specimen length addition (mm)

$L_1$  = Initial specimen length (mm)

### Water vapor permeability determination<sup>14</sup>

Water vapor permeability is analyzed by measuring water vapor transmission rate (WVTR) using wet cup method. Aluminium paper is cut with the proper size to close the

petri dish, and is then drilled in the middle for 10% of petri dish area. The hole made is covered with film whose WPV value will be measured. Then, petri dish filled with distilled water (around  $\frac{3}{4}$  part) is covered with aluminium paper prepared. Aluminium paper and petri dish are attached by epoxy adhesive, weighed, and then put on oven at  $37 \pm 0.5^\circ\text{C}$ . Sample is weighed every an hour for 5 hrs. The water weight loss is measured based on time function until steady state is achieved by using this formula.

$$\text{WVTR} = \frac{\text{Water weight loss at steady state}}{\text{Time} \times \text{Area}}$$

$$\text{WVP} = \frac{\text{WVTR} \times d}{S \times (R_1 - R_2)}$$

Remark: WVTR = Watervapor ransmission rate ( $\text{g s}^{-1} \text{m}^{-2}$ )

WVP = Water vapor permeability ( $\text{g s}^{-1} \text{m}^{-1} \text{Pa}^{-1}$ )

$d$  = Film thickness (m)

$S$  = Saturated air pressure at  $37^\circ\text{C}$  (6266.134 Pa)

$R_1$  = RH in petri dish (100%)

$R_2$  = RH at  $37^\circ\text{C}$  (81%)

### Thermal analysis<sup>15</sup>

Thermal stability and degradation profile of all chitosan films are determined by TGA. As much as 5 mg sample is introduced into high pressured stainless steel vessel. Sample is heated by rate  $10^\circ\text{C}/\text{mins}$  from dari  $20$ - $580^\circ\text{C}$  in nitrogen atmosphere. As much as 10 mg sample is then introduced into DCS aluminium vessel. Sample is then heated by  $10^\circ\text{C}/\text{mins}$  from  $20$ - $250^\circ\text{C}$  in nitrogen atmosfer. Data is analyzed by TASYs software.

### IR Spectrum determination<sup>15</sup>

IR spectrum of film is determinaed by FTIR spectrophotometer. Every spectrum is obtained from 16 times scanning at  $4 \text{ cm}^{-1}$  resolution and is operated at room temperature.

### Antimicrobial effectiveness assay by agar diffusion method<sup>11</sup>

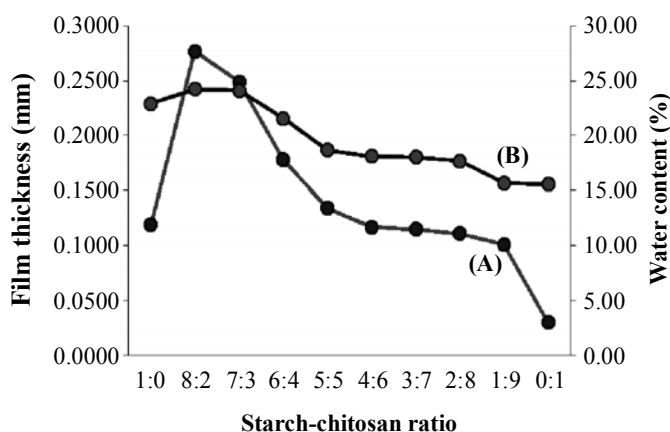
Agar diffusion method test antimicrobial activity based on inhibitory zone. Indicator culture used is *S. aureus* representing Gram positive bacteria and *E. coli* representing Gram negative bacteria. As much as  $100 \mu\text{L}$  inoculum solution is added into  $5 \text{ mL}$  liquid agar,

poured down to petri dish, and left until solidify. Film is cut as round shape (diameter  $\pm$  6 mm) and put above the agar prepared. Assay is conducted duplo for every film. The agar is then incubated for 24 hrs at 37°C (aerobe room for *E. coli*). Inhibitory zone diameter around the film is measured twice and is averaged.

## RESULTS AND DISCUSSION

### Thickness and film water content

Film thickness is measured by using micrometer at 5 different measurement points to obtain more accurate data. Thickness is required to know since it does affects tensile and water vapor permeability of a film. As the film thicken, the higher tensile and the lower water vapor permeability achieved. The films produced have various thicknesses. All of films are made by the same method, so that film thickness difference is caused by composing materials difference. Fig. 1, film having starch-chitosan 8:2 ratio is the thickest (0.277 mm), while chitosan film (control) is the thinnest (0.030 mm). Water content data obtained also in line with the hypotheses. The highest water content is possessed by the thickest film that is starch-chitosan 8:2 film, while the thickest chitosan film having the lowest water content. Water content of both films are 24.24% and 15.56%, respectively.



**Fig. 1: Thickness (A) and water content (B) of obtained film having various starch-chitosan ratio**

Film thickness increases as starch and glycerol content increment. It is caused by starch and glycerol hydrophilic property, so the film will absorb more water and the thickness increases<sup>16</sup>. Starch-chitosan 8:2 film is thicker than starch film (control) even though starch and glycerol composition in starch film (control) is higher. It is caused by

chitosan existence in starch-chitosan 8:2 film. Adding film composing materials component will affect thickness increment. The higher composing components, the film obtained will be thicker. Chitosan film (control) has the lowest thickness, even thinner than starch film. Chitosan property is less hydrophilic than starch as  $-OH$  functional group at C-2 change to  $-NH_2$  and  $-NHCOCH_3$ . Therefore, chitosan water-absorbing power is lower than starch.

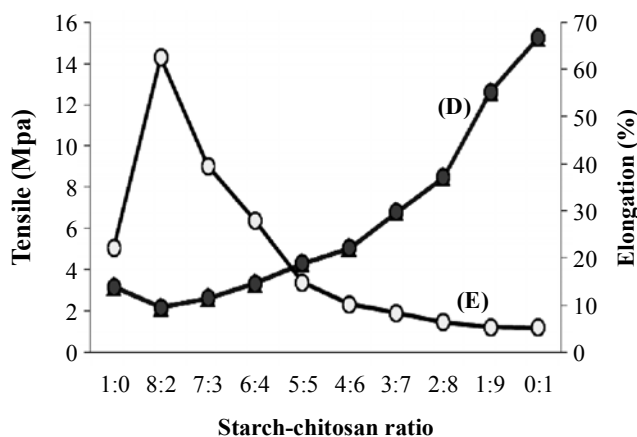
### **Film mechanical properties**

Biodegradable film mechanical properties are measured by pull test that will give tensile and elongation value. Tensile is a maximum tension given by film in retaining a load given before it breaks, while elongation is maximum length addition after pulling the film until break. The difference composition of starch, chitosan, and glycerol lies in contradictive effect in tensile and elongation value as showed in Fig. 2. The highest tensile value (15.23 MPa) is given by chitosan film, while the lowest tensile value (2.17 MPa) is given by starch-chitosan 8:2 film. The tensile value increases as chitosan ratio increment and glycerol amount decrement in film made. Starch has amylopectin content of 70-80% and amylose of 20-30%. Amylopectin has branched structure, while amylose has linear structure. Branches at amylopectin give difficulty in interacting to other amylopectin molecules, so it gives brittle property. Compared to amylose, linear structure of amylose ease the interaction between amylose molecules, so it strengthens the structure. Amylopectin content in starch is higher than amylose, so starch is tend to be brittle.

Combining starch with chitosan can strengthen starch film structure. It is proved from tensile value increment of film as chitosan ratio increases. Chitosan has linear structure like the cellulose, so the interaction to other molecules is easy. Chitosan will give additional interaction with starch molecule. Interaction occurred is ion-dipole interaction between  $-NH_3^+$  in chitosan chain and  $-OH$  in starch chain<sup>17</sup>. Amine functional group in chitosan is protonated in acetic acid solution to be  $NH_3^+$ , while regular crystalline structure in starch is deteriorated by gelatinization process, giving revealed  $-OH$  functional group, so it interacts with  $-NH_3^+$  in chitosan easily. That interaction will strengthen film structure. In film having very high starch ratio, tensile value increases insignificantly. It can be caused by intra-molecule hydrogen bond occurred in starch molecule. That interaction makes both of main components, starch and chitosan, do not interact each other.

Other than caused by starch ratio increment, glycerol concentration increment as plasticizer also has enough big role in decreasing tensile value of film made. Compared to other plasticizers, glycerol has enough small size, so it can penetrate between polymer chains easily. Hydroxyl functional group in glycerol will interact with hydroxyl group in starch and with amine in chitosan. This interaction will increase inter-polymer mobility and

cause polymers easy to slip each other, so the film achieved is more flexible<sup>18</sup>. As the glycerol concentration becomes higher, the obtained film becomes more flexible.



**Fig. 2: Tensile (D) and elongation (E) of film obtained from various starch-chitosan ratio**

In film having low concentration of glycerol (film with starch-chitosan ratio of 6:4, 5:5, and so on), the tensile value even surpasses starch film tensile. Liu et al.<sup>18</sup> reported that glycerol addition under 12% is able to make anti-plasticization, that is too strong interaction between plasticizer and polymer giving crosslinking effect, so the film become more rigid. Glycerol crosslinking with main polymer forming this film will decrease free volume in the system, so the polymer chain mobility will be decreased. After that, rearrangement of polymer network caused by strong interaction of plasticizer-polymer can cause crystallization, so the chain rigidity will increase or the chain flexibility will decrease.

Other than tensile value, elongation is also able to indicate film flexibility. The highest elongation value, that is 62.55%, is achieved from film having starch-chitosan 8:2 ratio, while the lowest elongation value, that is 5.25%, is achieved from pure chitosan film. Elongation value will be lower as chitosan composition increment in film made. Tensile is contrary to elongation. The greater its elongation, the smaller it's tensile, and the obtained film will be more flexible.

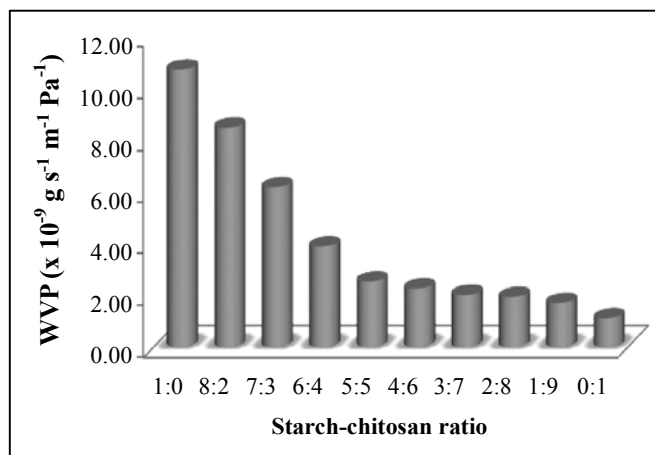
### Water vapor permeability

Water vapor permeability (WVP) visualizes material ability to pass water vapor at one area unit. Material WVP value can be affected by pore size and material hydrophilicity. The lower WVP value, the film quality is better. Film WVP values at various starch-chitosan ratios are showed in Fig. 3. Starch film WVP value (control) is  $1.07 \times 10^{-8} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ .



WVP value decreases as chitosan composition in the film increases. The lowest WVP value is achieved from chitosan film (control) that is  $1.10 \times 10^{-9} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ . The higher hydrophobic property of chitosan compared to starch contributes to this WVP value decrement. The more hydrophobic film composing materials, the more difficult water vapor to transmit through the film<sup>19</sup>. Besides,  $-\text{OH}$  functional group at starch will tend to interact more with  $-\text{NH}_3^+$  at chitosan than interact with  $-\text{OH}$  functional group at water molecule. That ion-dipole interaction decreases hydrophilic groups availability in film to interact with water vapor. Therefore, WVP value decreases as chitosan ratio in film increases<sup>20</sup>. This statement is also consistent with water content data. The higher chitosan composition in film, the smaller water content in the film.

Glycerol addition to film also gives effect to film WVP value. Based on Fig. 3, WVP value increases as glycerol composition in film increases. The similar result was reported by Chillo et al.<sup>21</sup> that glycerol addition increases film WVP. This is caused by the high glycerol hydrophilic property having 3  $-\text{OH}$  groups, which is able to form hydrogen interaction with water molecules easily. Hence, water molecules transmit through film easily. Besides, glycerol also decreases ion-dipole interaction of polymer inter-molecules, so there is distance between polymer molecules. It will decrease the density and increase free volume at film enlarging access for water molecules to transmit<sup>22</sup>. Therefore, hygroscopic plasticizer addition will always increase film WVP value.



**Fig. 3: Film WVP value achieved from various starch-chitosan ratios**

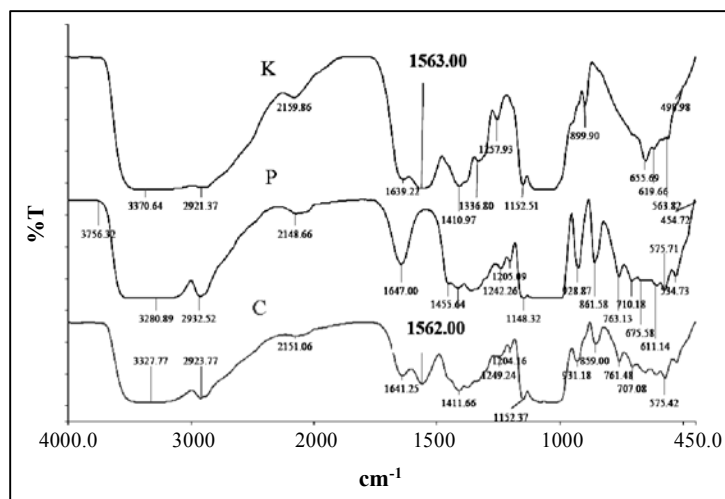
Starch type used to create film also determines WVP value. Corn starch has high enough amylose content, around 25–30%. Linear chain structure makes  $-\text{OH}$  group in amylose more accessible to water molecules than in amylopectin having branched structure.

As an impact, the higher amylose content in starch, the higher film WVP value achieved<sup>22</sup>. Some former research results reported lower WVP value for film made from other starch sources having lower amylose content than corn starch. Cassava starch film (amylose content is 19-25%) has WVP value of  $7.25 \times 10^{-11} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ <sup>23</sup> and wheat starch film (amylose content is 18-24%) has WVP value of  $4.00 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ <sup>24</sup>.

Lauric acid addition to film is also able to decrease film WVP value. WVP value of starch-chitosan 5:5 film made without adding lauric acid is  $2.88 \times 10^{-8} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ . If lauric acid as much as 8% from starch weight is added, the achieved film WVP value become lower, that is  $2.51 \times 10^{-8} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ . Lauric acid has 12 carbon atoms linear chain. This chain is hydrophobic, so it will hinder water molecules to transmit through film.

### Starch interaction with chitosan based on FTIR spectrum

The FTIR spectrum analysis is used to determine interaction between chitosan and starch in the film. Starch-chitosan 5:5 film is chosen to analyze through FTIR since it has mechanical properties representing all of film ratio made, that is optimum tensile and elongation between starch and chitosan film. Spectrum of starch film, chitosan film, and starch-chitosan 5:5 film are showed in Fig. 4.



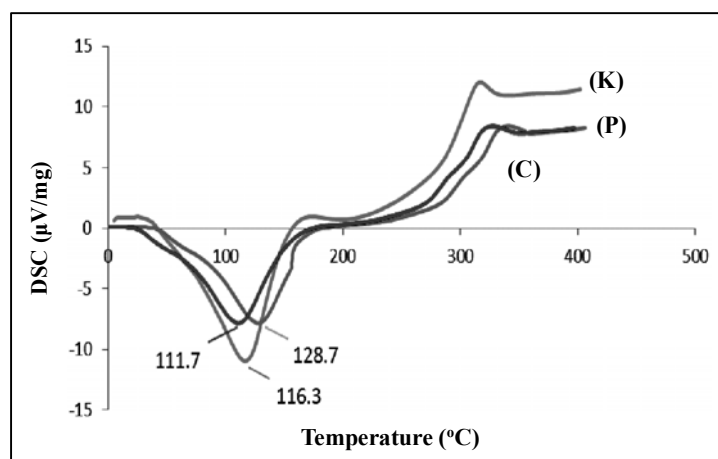
**Fig. 4: FTIR spectrum of chitosan film (K), starch film (P), and starch-chitosan film 5:5 (C)**

Three of them show O-H broad stretching peaks at 3200-3400  $\text{cm}^{-1}$  region, C-H small stretching peak at 2920-2940  $\text{cm}^{-1}$ , and O-H bending peak at 1630-1650  $\text{cm}^{-1}$ . In chitosan film, N-H stretching peak is covered by O-H stretching peak since both of them lie

in the same wavenumber region. However, N-H bending peak (amide II) is observed at  $1563\text{ cm}^{-1}$ . In the starch-chitosan film spectrum, amide peak shifts a bit from  $1563.00\text{ cm}^{-1}$  to  $1562.00\text{ cm}^{-1}$ . This shift indicates the interaction between  $-\text{OH}$  group in starch and  $-\text{NH}_2$  group in chitosan. Hydroxyl group absorption peak cannot be used to evaluate interaction between chitosan and starch as the glycerol and water also have hydroxyl group<sup>17</sup>.

### Film thermal property

Thermal analysis is a physical measurement method to evaluate polymer endurance and stability against temperature<sup>25</sup>. DSC instrument is used for film thermal analysis in this research, and giving thermogram of starch, chitosan, and starch-chitosan 5:5 film as showed in Fig. 5. Starch film shows endothermic peak at  $111.7^\circ\text{C}$  indicating film melting point. The chitosan film has higher melting point that is  $128.7^\circ\text{C}$ . Melting point at DSC thermogram shows polymer density. As the polymer density increases, interaction between those polymers will be stronger giving impact to the higher melting point. Chitosan has tough and solid linear structure, while starch has lower density. Amylopectin presence dominance makes whole structure of starch filled by many empty spaces. That causes polymer structure density decrement<sup>18</sup>. Therefore, starch has lower melting point than chitosan.



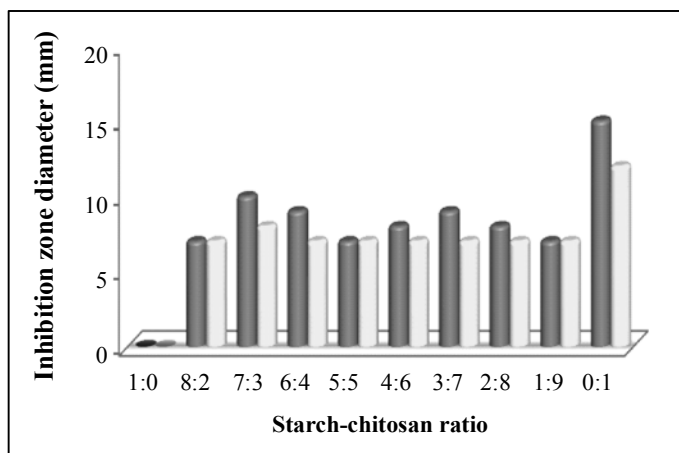
**Fig. 5: DSC thermogram of chitosan film (K), starch film (P), and starch-chitosan 5:5 film (C)**

Starch-chitosan 5:5 film has melting point of  $116.3^\circ\text{C}$ . This value lies between starch and chitosan melting point. It is caused by physical interaction between both of polymers. Ion-dipole interaction between ammonium group in chitosan and hydroxyl group in starch causes rearrangement of polymer chain framework. Starch polymer has a lot of cavities that will be filled with chitosan polymer when mixed with chitosan and new interaction between

starch polymer and chitosan polymer occurs. This interaction gives tougher polymer structure than starch film since the empty cavities decrease. Yet, it is more brittle than chitosan film since it stretches chitosan polymer lattices<sup>26</sup>. It causes starch-chitosan melting point lies between starch film and chitosan film melting point.

### Film antimicrobial property

Antimicrobial property analysis is conducted to evaluate film strength to inhibit bacteria growth around. Antimicrobial activity of starch, chitosan, and starch-chitosan film is showed in Fig. 6. Starch film does not show antimicrobial activity against both of bacteria tested, indicating from inhibition zone absence. Most of films has broader *S. aureus* inhibition zone than *E. coli*. The longest inhibition zone diameter for *S. aureus* and *E. coli* is achieved by starch-chitosan 7:3 film, 10 and 8 mm, respectively. Film having starch-chitosan 8:2, 5:5, and 1:9 ratio have same inhibition zone diameter for both bacteria that is 7 mm. This result is different with Saleh and Muhamad<sup>11</sup> research showing that the broadest inhibition zone of Gram positive bacteria is achieved by starch-chitosan 8:2 film with diameter of 11.3 mm, while for Gram negative bacteria is achieved by starch-chitosan 9:1 film with diameter of 10.5 mm.



**Fig. 6: Antimicrobial activity of film achieved from various starch-chitosan ratio: *S. aureus* (■), and *E. coli* (□)**

In starch-chitosan film, chitosan has big role in antimicrobial activity. Ammonium group in chitosan can interact with bacteria cell outer membrane having negative charge. Interaction of those different charges triggers bacteria cell lysis<sup>27</sup>. Gram negative bacteria cell is composed from peptidoglycan thin membrane, while the outer membrane consists of lipopolysaccharide, lipoprotein, and phospholipid. Ammonium group can interact with

carboxyl anion and phosphate group in lipopolysaccharide and phospholipid. It causes film containing chitosan has antimicrobial activity against Gram negative bacteria, *E. coli*<sup>28</sup>.

The similar mechanism is also occurred on Gram positive bacteria, *S. aureus*. A gram positive bacterium has thick cell wall composing from peptidoglycan thick membrane and teichoic acid polymer. Teichoic acid main chain has a lot of phosphate groups, so it is very negatively charge and is able to gives electrostatic interaction with cationic antimicrobial compound such as chitosan salt<sup>29</sup>. Anionic group amount in Gram positive bacteria is higher than Gram negative bacteria. It causes film antimicrobial activity in Gram positive bacteria, *S. aureus* is tend to be higher.

Bacteria growth inhibition response is classified into very strong (inhibition zone diameter >20 mm), strong (16-20 mm), moderate (10-15 mm), and low (< 10 mm)<sup>30</sup>. Based on this classification, only starch-chitosan 7:3 film having moderate inhibition response against *S. aureus*, while the other films have low inhibition response. In the other side, all films are classified into low inhibition response against *E. coli*.

## CONCLUSION

Starch-chitosan-lauric acid film was successfully made by various starch-chitosan ratios. Film has yellow color and become darker as starch composition increases. Chitosan ratio increment increases film tensile value and also decrease elongation and WVP. Starch-chitosan film has melting point between starch and chitosan film melting point. FTIR spectrum shows that ion-dipole interaction between ammonium group in chitosan and hydroxyl group in starch is occurred, which is seen from N-H bending peak shift in starch-chitosan film. Starch-chitosan 7:3 film has the best antimicrobial activity having moderate inhibition response to Gram positive bacteria and low inhibition response against Gram negative bacteria. This film is not feasible yet to be marketed as its mechanical properties and antimicrobial activity are still below the standard.

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*Accepted : 25.01.2016*