THE CHARACTERISTIC OF SAGO-BASED EDIBLE FILMS INCORPORATED WITH DIFFERENT ANTIMICROBIAL ESSENTIAL OILS

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***Abstract*—Sago films incorporated with essential oils (EOs) was produced to study their characteristics and antimicrobial effectiveness against *Escherichia coli*. The films were prepared by heating a mixture of sago starch and glycerol as plasticizer. Two concentration of glycerol were used, 0.6 and 1.0 mL with an incorporation of antimicrobial agent such as turmeric (TO) and lemongrass (LGO) EO to film mixture with different concentration (0.2%, to 0.5% v/v). Tensile testing machine was used to measure the tensile strength (TS) and elongation at break (EB) of the sago film. Other characterization including water vapor permeation (WVP), antimicrobial activity and color of the films. The sago film with 0.6 mL amount of glycerol for both EOs incorporated have slightly increased the tensile strength and elongation at break as concentration of EOs increased. WVP for 0.6 mL glycerol film with both EOs have increased compare to film with 1.0 mL glycerol which having the decreasing amount of WVP as the concentration of EO increased. Antimicrobial activity was test against *Escherichia coli* (*E.coli*). Result showed that sago film with LGO at 0.2%v/v has the lowest total colony count for both amount of glycerol added to films. Both films with 0.6 mL and 1.0 mL glycerol incorporated with LGO and turmeric have a significant yellow color compared to commercialize ziploc sandwich bag bought from supermarket.**

Keywords*— antimicrobial activity, color, EB, edible film, essential oil, Escherichia coli, glycerol, LGO, sago starch, turmeric, TS, water vapor permeability.

I. INTRODUCTION

Conventional packaging such as plastic is commonly used in food packaging since many years ago. The usage of plastic as food packaging have become a concern issue as it is non degradable and have to undergo heat treatment which can affect environment due to toxic emit. A lot of researchers have done their studies in improving the usage of degradable packaging by producing edible film which have various advantages such as extending food shelf life, protecting food from food contamination and other factors such as moisture, gases and lipid migration. It also can be part of supporters of additives and nutrients (Campos *et al*. 2011). Edible film is derived from a range of naturally material which can be divided into three categories hydrocolloid lipids and composite. Starch, alginate, chitosan and cellulose derivatives are classified as polysaccharides group while lipids include waxes, acylglycerols and fatty acids. Composite is derived from the combination of those group of material (Du *et al*. 2011).

In this research sago-starch is used to produce film. Sago starch is an abundant source that have been greatly dispersed throughout South East Asia was isolated from sago palm in the genus of *metroxylan spp.* (Ahmad *et al.* 1999). Starch has good film-forming ability due to its unique colloidal properties. Besides, low cost of raw material along its wide availability and ease of handling have make starch as the most common raw material used in edible film formation (Maizura *et al*. 2008). Glycerol is commonly added to the film formulation as plasticizer to modify the mechanical properties by reducing an intermolecular hydrogen bonding between polymer chain with increasing molecular volume forming a great film flexibility (Mali *et al.* 2006).

Incorporation of Essential oils which extracted from plant such as turmeric, lemongrass, ginger and others have concentrated hydrophobic liquid containing volatile aroma compounds can be added into the edible film (Avila-Sosa *et al*. 2012). EOs are usually inherent antimicrobial activity and aroma that can used as additives and generally recognized as safe (GRAS) (Tunc *et al*. 2007).

The objective of this study were to characterize the mechanical and barrier properties of the sago based film incorporated with different antimicrobial essential oils. The effectiveness of antimicrobial activity of these films against *E.coli* was also investigated.

II. METHODOLOGY

A. Materials

Sago starch (*Metroxylon sagu*) was purchased from Amcee Food Industries Sdn. Bhd (Kuantan, Pahang). Other chemicals used for this film preparation include glycerol and ethanol with concentration of 95% which obtained from Chemistry Laboratory, Faculty of Chemical Engineering that supply by Classic Chemical Sdn. Bhd, pure extracted lemongrass and turmeric EO were purchased from Young Living Malaysia Sdn. Bhd (Kuala Lumpur, Malaysia) and LB broth and Nutrient agar were purchased from Merck, (Darmstadt, Germany). *E.coli* that have been cultured from pure *E.coli* were obtained from Bioprocess Laboratory of Faculty of Chemical Engineering Uitm Shah Alam.

B. Preparation of Raw Material

Sago starch edible film was prepared by dissolving 3 g of sago starch into 120 mL of distilled water. The starch solution was heating on hot plate at 500 rpm until it reach 75°C. Then 0.6 mL of glycerol was added to the solution and continued to heat for 30 min until gelatinization occurred. Dilution of lemongrass oil (LGO) was initially prepared to 10% concentration with the addition of 95% ethanol by the ratio of 1:9 of lemongrass to ethanol (Maizura *et al.* 2008). The gelatinized sago solution was cooled to room temperature before the diluted of lemongrass added at different concentration (0.2%, 0.3%, 0.4% and 0.5% v/v of film forming solution) with continuous stirring to ensure homogeneous solution. The solution was casted onto petri dish with uniform volume of 30 mL and lastly dried in oven at 40°C for 24 hr. Another film solution with the same formulation and concentration of lemongrass EO was prepared but having a different amount of glycerol added which was 1.0 mL as comparison. Those two set of variable were applied to another antimicrobial EO, turmeric oil (TO) to differentiate the effect the incorporation of those EOs into sago based edible film.

C. Organism and Preparation of Cultures

E.coli culture was obtained from the Bioprocess Laboratory, Faculty Chemical Engineering of UiTM Shah Alam, Malaysia). Nutrient agar has prepared as a medium for the *E.coli* culture to grow and kept at 4°C for further used. *E.coli* was subculture in LB broth to maintain the culture viability.

D. Mechanical Properties

Tensile strength and elongation at break of sago starch film were examined using Tensile Testing Machine (Tinius Olsen) with load of 2.5kN. Three sample of each formulation was cut (5.0 × 2.0 cm) and measured it thickness before those were clamped between the tensile grips. Each film strip was placed in pneumatic grips and stretched at 50 mm m^{-1} with an initial distance between grips of 30 mm. This method was according to (Bajpai *et al*. 2011) with a modification and without extensometer grip. The parameter that should be consider is tensile strength (MPa) and elongation at break (%) with three replicate were being tested for each films.

E. Water Vapor Permeation

Water vapor permeation was carried out by using method described by (Rangel-Marrón *et al*. 2013). Each film samples with different concentration of different EOs were cut into 5 diameter, placed on the top of glass jar and sealed using parafilm. Each of the glass jar was initially have been filled up with 5 mL of distilled water before being sealed. There must be a gap between surface of water and the film for an air space. Then the glass jar samples were stored in desiccator containing silica gel at 33% RH. Weight of the glass jar with samples are measure for every 1 hour for 8 hours periods. The first data needed was water vapor transmission rate (WVTR) which can be obtained by plotting the slope of the regression analysis of weight loss as function of time (g/s) then divide by the exposed area of the film to get water vapor permeability which can be expressed by the following formula:

$$WVTR = m_l/A = g/m^2s \quad (1)$$

$$WVP = L \times WVTR/ (p_l - p_a) \quad (2)$$

Where m_l is the slope of graph plotted, A is exposed area; p_l and p_a are partial pressures of water vapor in the air and air saturated respectively to 33% RH at 25°C while L is the average thickness of the films.

F. Antimicrobial Activity

Antimicrobial activity test on the films was carried out using the agar diffusion method as used in the study by A.A Karim and others (2007). The total colony formed on the agar medium was counted for determination of antimicrobial effect of the film against *E,coli* colony. The *E.coli* culture was initially undergo a serial dilution with peptone water to dilution factor of six (10^6 CFU/mL). Then 0.2mL of the diluted culture was inoculate to the nutrient agar before 6mm diameter of sago based edible film with different concentration of TO and LGO was placed on the nutrient agar with *E.coli* culture.

G. Color

The color of the film incorporated with different EO affected the native color of edible film and the color was examined by using CR-400 Chroma meter (Kinoca Minolta) The chromameter was put closely on the films samples and reading L*, a* and b* value was taken (Ghasemlou *et al*. 2013). L* value indicate the lightness of sample where the highest value of L (100) indicate white and the lowest value (0) indicate black. For a* value, it point to the redness of the product. The colour range is from green (-60) to red (+60) while b* values be a sign of blueness of product. It range from blue (-60) to yellow (+60). A standard commercialize sandwich bag plastic was used to compared the changing color of increasing concentration EOs added to each films. ΔE index which indicate the estimated color difference between samples and standard was calculated by following equation:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (3)$$

Where L*, a* and b* are the color value for sago film incorporated with both EOs while L, a and b are the color parameter for standard plastic.

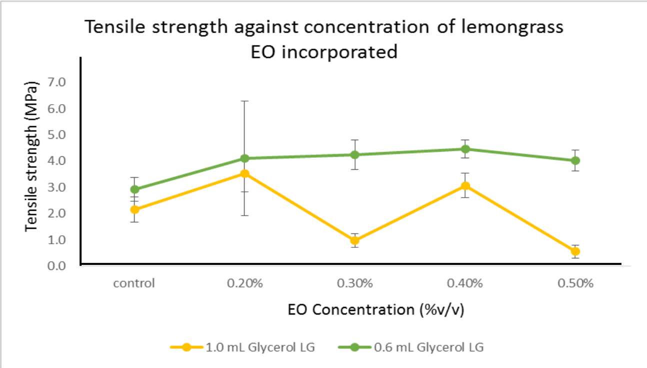
III. RESULTS AND DISCUSSION

A. Mechanical Properties

The amount of glycerol added to the film which act as plasticizer as well as the incorporation of EOs on the films with different concentrations have shown a significant different in mechanical properties from the control sago film. Generally positive results obtaining using sago film with 0.6 mL glycerol as the film was showing an increased TS compared to film with 1.0 mL glycerol. In Figure 1, TS for sago film with 0.6 mL glycerol LGO was slightly increased as the concentration of LGO increased, similar to the film with 0.6 mL glycerol TO which reflecting the finding by Dani Supardan *et al*. (2016). However the result was supported by Wen-Xian Du *et al.* (2011) which also having an increasing value of TS, EB and modulus of the apple based film that incorporated with EOs. This finding was related to the different in polarities between the films and EOs incorporated. Results obtained also similar to Pranoto, Salokhe, and Rakshit (2005) whom studied about the physical and antibacterial properties of alginate edible film with garlic oil.

Figure 2 was showed the film with 1.0 mL glycerol have slightly decreased of TS along the increased TO concentration as well as for LGO film with 1.0mL glycerol. The film with 1.0 mL glycerol for both type of EO incorporated have reduced the TS of film significantly which resulted from the typical plasticizing effect of glycerol that make it too soggy (A.Fazilah *et al.* 2007). Addition of EO also showing an significant factor of decreasing tensile strength may be due to the complex structure between starch polymer and lipid part from the oil that reduces the cohesion of the starch network forces thus decreasing the films resistance to breakage (Ghasemlou *et al*. 2013).

(a)



(b)

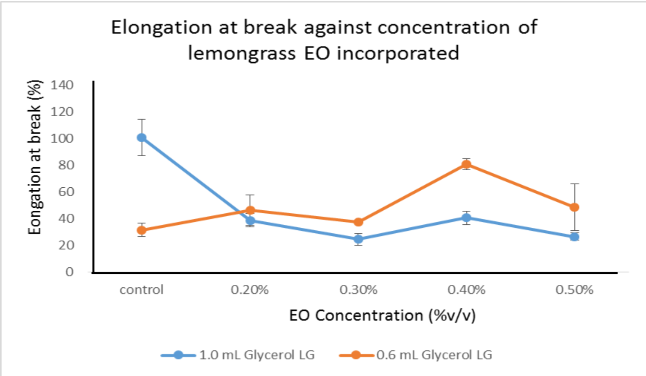
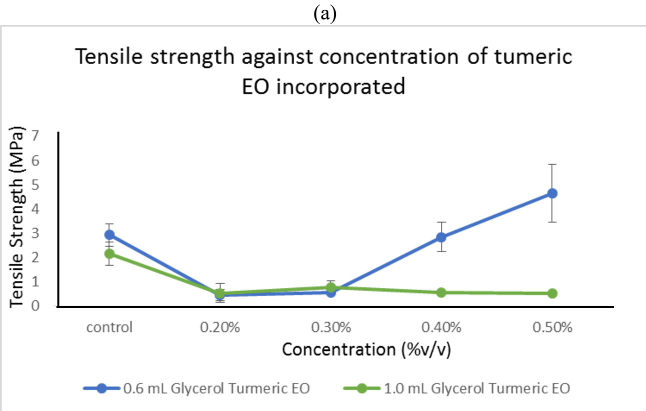


Fig. 1 Tensile strength (a) and elongation at break (b) for 0.6 and 1.0 mL glycerol of sago film incorporated with lemongrass essential oil.

(a)



(b)

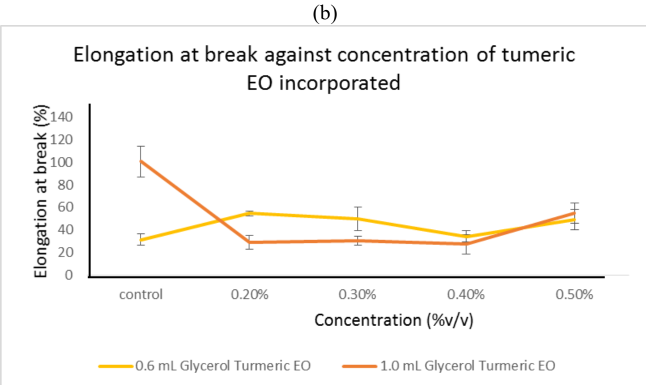


Fig. 2 Tensile strength (a) and elongation at break (b) for 0.6 and 1.0 mL glycerol of sago film incorporated with turmeric essential oil.

All of the films in this study showing a decreasing value or not significantly changes as concentration increase instead of increasing EB which as typically reported by others. This may due to the glass transition (T_g) properties of the film which was closed to testing temperature (room temperature). The glass transition temperature of resin system can be define when thermoset polymer undergoes changes from an amorphous rigid state to more flexible state which depends on the nature of the polymer at its service temperature. As for this sago films it become rigid and inflexible when it under that condition resulting in the insignificant changes or decreasing of EB (A., M., and M.H. 2007)

B. Water Vapor Permeation

Water vapor permeation test was performed to study how good the films can resist the moisture permeation of food product to surrounding. The WVP of the film should be as low as possible since the ultimate function of food packaging is to prevent or at least reduce the moisture transfer between food and the surrounding atmosphere. Result of water vapor permeation of all film were shown in Figure 3.

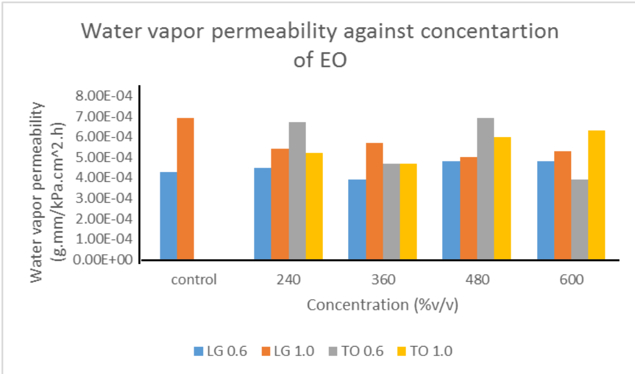


Fig. 3 Water vapor permeability of sago films with 0.6 mL and 1.0 mL glycerol incorporated with lemongrass and turmeric essential oil.

Figure 3 show that water vapor permeation for each EO with different amount of glycerol. Film with 0.6mL glycerol give a lower water vapor permeation value compared to film with 1.0mL glycerol which indicates that the amount of plasticizer affects the moisture barrier of the film. Addition of LGO and TO seems to be helpful in maintaining the barrier properties of the films which showing a slightly decreasing of WVP. This is due to the hydrogen and covalent between the starch network and the polyphenolic compounds of the EO. The availability of hydrogen group to form hydrophilic bonds with water interaction was limited that lead to a decrease in film's affinity for water (Ghasemlou *et al*. 2013).

C. Antimicrobial Activity

LGO incorporated sago films have shown significant inhibition of *E.coli* compared to TO incorporated films. For both glycerol concentrations of 0.6 and 1.0 mL, the addition of 0.2% v/v LGO gave the lowest total plate count of 8.09 CFU/mL and 7.82 CFU/mL respectively. The antimicrobial agent effectiveness was measured by the amount or concentration of substance added. Theoretically, lemongrass has an important component that called citra an acyclic unsaturated monoterpene aldehyde compound which may damage the cell membrane that lead to microbe inhibition. However, the compound is highly volatile and sensitive thermally, similar to TO (Dani Supardan et al. 2016). No significant inhibition was observed for films with EOs more than 0.2% v/v. This may due to the inability of EO to be released from the saccharides matrix of sago that reduced antimicrobial activity as compared to directly pour of antimicrobial oil.

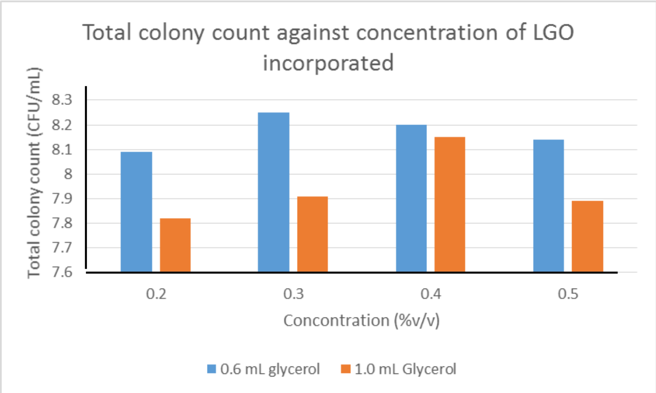


Fig. 4 Antimicrobial activity of sago film incorporated with lemongrass essential oils based on total colony count.

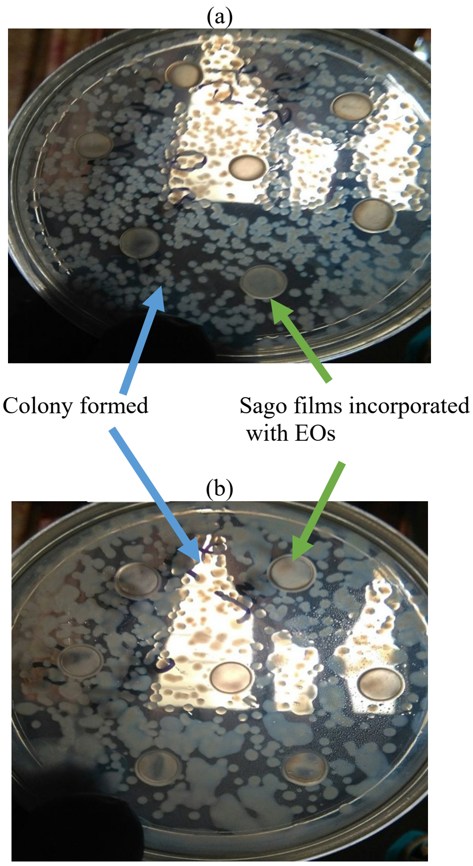


Fig. 4 Growth of *E.coli* colony on nutrient agar that containing sago film incorporated with LGO at 0.2 % v/v concentration for 0.6 mL glycerol (a) and 1.0 mL glycerol (b)

Incorporation of sago based films with TO was not commonly studied by researchers. Tumeric is one of the popular antimicrobial agent in food industry however in this study TO have shown a very low antimicrobial activity where the inhibition of *E.coli* on the film is very low. This may be due to the formulation of the tumeric incorporated into sago based film. The TO that has incorporated into sago film was initially being diluted with 95% ethanol to get 10% concentration which was a modification method state by (Maizura *et al*. 2008) so the concentration of the pure TO was reduced. Furthermore, ethanol and TO is a very volatile substance. It has been reported that starch and chitosan films reserved volatile components present in the essential oil less efficiently since it only has one type of polymer chain (Dani Supardan *et al*. 2016). Thus it allowed more volatile components into vapor phase which resulting in decrease of antimicrobial activities of the film. The incorporation of the TO and the sago solution also could be the problem as the homogenous solution were hard to be reach as there is no emulsifier agent or other crosslinking agent added to the formulation.

D. *Color*

Product appearance play an important role in influencing consumer acceptability. One of the major aspect of product appearance is the color of the film. Incorporation of EO in edible film may alter the natural color of the edible film. The color of all the film incorporated with different concentrations of LGO and TO were measured by chromameter. The color of the films were compared to the standard which was sandwich bag ziploc. Table 1 and 2 was shown the parameter value of L*, a* and b* measured by chromameter value of films with ΔE that calculated by using equation (3) to standard value of commercial plastic. The significant changes of L value resulted a similar trend to ΔE. The results obtained are in agreement with the findings of a study by (Ghasemlou *et al*. 2013) where the sago films become more opaque with the incorporation of essential oils. Increased in diffuse reflectance provoked by light scattering in the lipid droplet which lower both the light scattering intensity and the film's whiteness index.

Table 1. L*, a* and b* value of films incorporated with lemongrass EO and total color difference with the standard

Amount of glycerol	Film type	EO Conc (%v/v)	L*	a*	b*	ΔE	Std Dev
	Control	0	31.33	-0.16	0.16	-10.29	0.13
0.6 mL	LGO	0.2	32.68	-0.97	0.41	-9.5	0.27
	LGO	0.3	32.55	-1.32	0.41	-9.98	0.52
	LGO	0.4	32.73	-1.01	0.21	-9.69	0.44
	LGO	0.5	32.91	-0.92	0.28	-9.35	0.33
1.0 mL	LGO	0.2	33.99	-0.36	0.58	-9.5	0.83
	LGO	0.3	33.88	-0.34	0.10	-9.98	0.10
	LGO	0.4	33.75	-0.29	0.24	-9.69	0.30
	LGO	0.5	33.60	-0.37	0.26	-9.35	0.31

Table 2. L*, a* and b* value of films incorporated with turmeric EO and total color difference with the standard

Amount of glycerol	Film type	EO Conc (%v/v)	L*	a*	b*	ΔE	Std Dev
	Control	0	31.67	-0.33	0.14	-10.29	0.11
1.0 mL	TO	0.2	31.12	-0.22	0.17	-8.67	0.04
	TO	0.3	31.68	-0.15	0.20	-6.95	0.25
	TO	0.4	31.69	-0.32	0.27	-6.87	0.20
	TO	0.5	32.01	-0.16	0.15	-6.53	1.48
0.6 mL	TO	0.2	33.18	-0.57	0.34	-7.41	0.28
	TO	0.3	34.50	-0.37	0.54	-7.98	0.04
	TO	0.4	34.65	-0.23	0.33	-7.92	0.09
	TO	0.5	34.55	-0.54	1.08	-8.13	0.05

IV. CONCLUSION

Essential oil such as lemongrass and turmeric that have been incorporated on sago film have shown a significant different in physico-mechanical properties. They have better tensile strength and elongation at break. Incorporation of essential oils also increase the moisture barrier properties of the films and have the potential to inhibit growth of spoilage microbes.

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