Development of Chitosan Blended Adlay Starch Films for Antimicrobial Packaging to Extend Shelf Life of Fruits

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ABSTRACT

The objective of this research was to study the forming of adlay starch film. The glycerol concentrations of 10, 15 and 20% (w/w, starch) were tested to determine an optimal one to be used as plasticizer. The glycerol was added into the dispersions at physical properties including thickness, tensile strength, elongation value and biodegradation were evaluated. The results indicated that adlay starch with 10% glycerol showed the maximum tensile strength of 0.34±0.03 MPa while the elongation value of this condition was the lowest at 36.52±1.05 mm. When glycerol concentration was raised to over 20%w/w, it presented the lowest tensile strength (0.08±0.01MPa) with an elongation that reached 53.07±5.92 mm. The adlay starch film with 20% glycerol was completely degraded within the shortest time period of 14 days. For the antimicrobial activity of films mixed with different chitosan concentrations, the greatest antimicrobial activity against three bacteria tested was the effect of starch films containing 1.5 and 2.0% of chitosan. Moreover, the results indicated that bananas packed into starch films with 1.5% and 2.0%w/w chitosan demonstrated their shelf life extension until to 14 days.

Keywords: Biofilm, Adlay starch, Chitosan, Antimicrobial packaging

INTRODUCTION

Food storage containers for food packaging are normally used for protecting foods from environmental influences such as dusts, moisture, light, air, insects, etc. (Arvanitoyannis & Oikonomou, 2012; Pereira de Abreu et al., 2012). However, some of packaging cannot prevent microbiological contamination that causes food spoilage and fails to extend the shelf life of food products. Therefore, packaging technologies are of interest with the aim to develop an active packaging or antimicrobial packaging which is designed for increasing the shelf-life of food products by prevention of the microbial contamination. The active packaging acts by releasing antimicrobial agents in the structure of packaging materials to inhibit bacterial growth on the surface of food (Gharsallaoui et al., 2016).

Biopolymers or bioplastics are generally used as raw materials for active packaging production due to their biodegradable and environmentally friendly properties. Thus, it becomes a viable alternative way to replace synthetic plastics in food packaging application. Most studies have suggested various biological resources like starch, cellulose, sugar and proteins for active packaging applications

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(Nicosia et al., 2015; Tawakkal et al., 2016). Starch is the most important biopolymer used as biobased packaging material to develop biodegradable films. It is commonly found in many plants such as corn, tapioca, cassava, potatoes, wheat, rice, etc. (Gadhave et al., 2018) In addition, starch-based plasticizers improve flexibility of the films (Vieira et al., 2011). Antimicrobial agents can be inserted homogeneously to the structure by coating and immobilization of the agents to the surface of the packages. Therefore, it is suitable material to prepared active packaging.

Antimicrobial agents such as some organic compounds (a-tocopherol, nisin or benzoate), enzymes and nanoparticles (titanium dioxide, and zinc oxide)) are important components of active food packaging that show microbial activities and several are widely used in active packaging such as some organic compounds (Amini et al. 2014). However, most of them are the synthetic preservatives that lead to the presence of residual chemicals in food products (Wessling et al., 1998) Therefore, for the safety of consumers, the bioactive components are considered as more suitable packaging materials than synthetic ones. Chitosan extracted from shrimp and crab shells has been approved to be nontoxic and biodegradable. It also represents the antimicrobial activity against some microbes (bacteria, yeasts and fungi) (Goy et al., 2009). Therefore, this study used chitosan as an antimicrobial agent in packaging and aimed to investigate its antimicrobial activity.

This research was aimed to develop an antimicrobial packaging biofilm from adlay starch-chitosan blend antimicrobial film. The antimicrobial activity of the bioactive compound was tested against some pathogenic bacteria (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* DMST 15537 and *Pseudomonas aeruginosa* ATCC 27853). In addition, the optimal film will be further applied to extend the shelf life of fresh fruits.

MATERIALS AND METHOD

Material collection and sample preparation

Adlay starch was prepared by using the dry-milling process modified from method of Capule and Trinidad (2016) to obtain the particles that passed through 80 mesh sieves. The adlay starch base film was produced by gelation with the solution-casting method. Initially, starch was dissolved in distilled water to prepare starch solution at concentration of 20% (w/v) under constant stirring for 10 min at room temperature (25˚C). Different concentrations of glycerol were used as plasticizer to investigate its effect on film properties. Glycerol was added into the dispersions at 10, 15 and 20% (w/w, starch). The mixtures were heated at 80˚C under constant stirring for 20 min to convert into gelatinous form, and then cooled to 40˚C. The mixtures were casted onto 10x10 cm^2 flat sheet film mold to set and dried overnight at 55˚C for 24 h, and then cooled to ambient temperature. The dried sheet film was peeled off from the mold and stored in a desiccator at room temperature for further analysis.

Film properties testing

The physical properties of film including thickness, tensile strength, elongation and biodegradation were determined.

Film thickness

The films were cut into a square shape of 9 X 9 cm. The film thickness was measured with a digital micrometer (IP65 Mitutoyo, Japan) according the standard method, ASTM D 645-92 (ASTM 1966) at five positions on the film including the center and other four opposite positions. All measurements were performed in triplicate. The average film thickness was calculated.

Tensile strength and elongation at break (TS and E).

Tensile strength test was based on the standard method ASTM D 882-88 (ASTM 1989) by using the universal testing machine (Instron Engineering Corporation, Canton, MA). All film samples were prepared by cutting into dumbbell shape (Figure 1). An initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively.

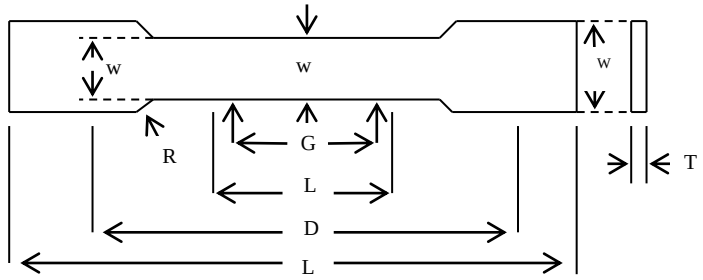


Figure 1 The film sample cut into dumbbell shape

Each of film samples was fixed between two tensile grips and the gauge length was set at 5 cm. The film was pulled to separate by the tensile grips using a constant rate of speed. Tensile strength was analyzed according to elongation of the film before breaking. The tensile strength, percent elongation at break was calculated as average values.

Biodegradation properties

Four sheets of film were cut into 3 cm x 3 cm and then were buried inside the glass tank (24×12×14 cm) that contained soil. Each of film was buried inside the soil at a depth of 10 cm with 2 cm distance between them under room conditions. The degradation processes were investigated every 2 days for 14 days.

Preparation of chitosan blended adlay starch film

The film was prepared from 20% (w/v) starch solution with the optimal concentration of glycerol (from 1). Four different concentrations of chitosan (0.5 1.0 1.5 and 2 %w/v) were dissolved in 5% acetic acid solution and added into mixed starch-glycerol solution. The mixtures were heated at 80˚C under constant stirring for 20 min until they become homogeneous, and then cooled to 40˚C. The mixtures were poured to set onto 10x10 cm^2 flat sheet film mold and dried overnight at 55˚C for 24 h, and then cooled at room temperature. The dried films were peeled off from the mold and stored in a desiccator at room temperature for antimicrobial evaluation.

Antibacterial activity test of chitosan blended adlay starch film

Antibacterial activity of film was performed using agar diffusion method described by Maizura et al. (2007). The antibacterial activity of each film was tested against *S. aureus* ATCC 25923, *E. coli* DMST 15537 and *P. aeruginosa* ATCC 27853 derived from Science and Technology Service Center, Chiang Mai university (STSC-CMU). Each bacterial test strain was cultured in 5 ml Mueller Hinton broth (MHB; Difco Laboratories, USA) at 37˚C for 16-18 h and the standardized concentration which was equivalent to concentration based on 0.5 McFarland ($1x10^8$ CFU/ml) at OD_{600} nm. Subsequently, each strain was swabbed on Mueller - Hinton agar. Each of film was cut into 6 mm diameter and placed on MHA plate. Then MHA plates were incubated at 37 ˚C for 48 hrs. Unmixed chitosan - starch film was used as the negative control. Gentamicin (BIO BASIC INC.), 1 mg/ml was used for the positive control. The bacterial inhibition zone was examined on the film discs and reported in millimeter (mm). The experiment was determined in triplicate.

Effect of antibacterial films on shelf-life extension of bananas

Cavendish Banana (*Musa acuminate)* used as a sample of fruit in this study was collected from Ban Du fresh market, Chiang Rai, Thailand. The samples were packed into different packaging films; 1) petroleum plastic (LDPE plastic), 2) unmixed starch based film, 3) 0.5% chitosan-starch mixed film, 4) 1.0% chitosan-starch mixed film, 5) 1.5% chitosan-starch mixed film and 6) 2.0% chitosan-starch mixed film (Figure 2). The unpacked sample was used as control. Thereafter, all of samples were stored at ambient temperature. The changes in color of bananas were evaluated at the end of storage (day 14), according to the following numerical scale for scoring these attributes: 1) more green than yellow, 2) yellow with green tip, 3) all yellow, 4) yellow with spots (1-20%), 5) yellow with spots (21-50%), 6) yellow with spots (51-80%) and 7) all brown with black spots. Each sample was replicated 3 times.

Figure 2 Bananas coated with chitosan-starch mixed film

RESULTS AND DISCUSSIONS

Starch based films were produced by casting method using 20% adlay starch solution with adding of three levels of glycerol (10, 15 and 20%w/w, starch basis). After the solution was set on aluminum plate, the film was peeled off, as shown in Figure 3.

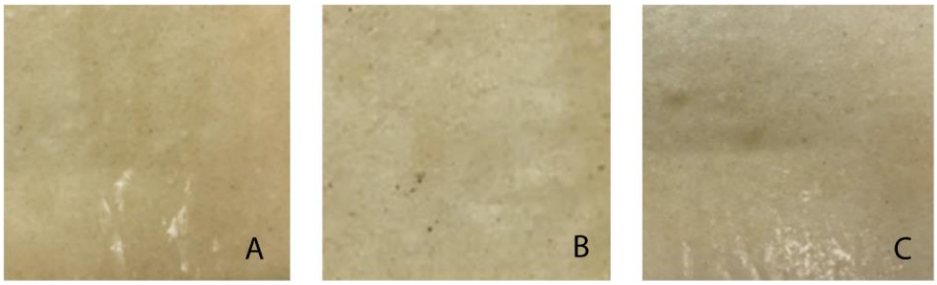


Figure 3 Adlay starch-based films, A**:** starch film mixed with 10% w/w glycerol, B: starch film mixed with 15% w/w glycerol, C: starch film mixed with 20% w/w glycerol

Film properties

The thickness of film was measured according to standard method, ASTM D 645-92 (ASTM 1966). Thickness of films made from different concentrations of starch and glycerol ranged from 0.76±0.04 to 0.92±0.05 mm. Measurement of tensile strength and elongation was based on the standard method ASTM D 882-88, as shown in Table 1 and mechanical properties of bioplastics in terms of tensile strength and elongation were presented in Figure 4 and 5.

Table 1 The physical properties of films

Glycerol (%w/w)	Thickness (mm)	Max. Force (N)	Tensile strength (MPa)	Extension (mm)	Elongation (%)
10%	0.76±0.04	3.1±3.10	0.34±0.03	36.52±1.05	73.04±2.10
15%	0.76±0.12	1.3±0.00	0.16±0.05	48.54±2.085	97.07±4.17
20%	0.92±0.05	0.9±0.17	0.08±0.01	3.07±5.92	106.14±11.85

Table 1 showed the mechanical properties of films that were prepared by varying concentrations of glycerol which was used as plasticizer (10, 15, 20% w/w, starch basis). The results of physical properties in terms of tensile strength (TS) and elongation percentages (E) were represented in Figure 4 and 5.

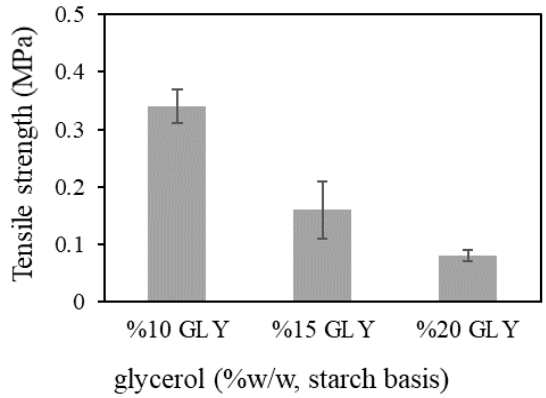


Figure 4 Tensile strength of film

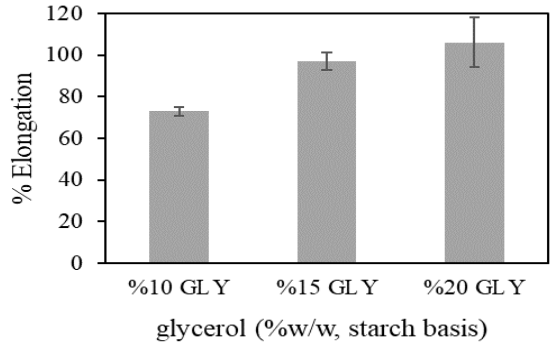


Figure 5 Elongation percentages of film

The results showed that increasing concentration of glycerol (plasticizer) resulted in decreased tensile strength. Tensile strength decreased from 0.34±0.03 MPa to 0.08±0.01 MPa when glycerol increased from 10% to 20% w/w. Addition of

plasticizer concentration can reduce the tensile strength of film due to weak molecular bonding in film caused by plasticizer. Moreover, the effect of plasticizer enhanced flexibility between adjacent molecules of the film (Jouki et al., 2013; Zanela et al., 2015; Basiak et al., 2017). Normally, plasticizer molecules have small molar mass thus they can mediate interaction between polymer molecular chains (Tapia-Blácido et al. 2013). Especially, glycerol was reported to be more efficient plasticizer than other plasticizers with its small size (92.09 g/mol). (Bourtoom 2008; Muscat et al., 2012; Razavi et al., 2105). This size of glycerol supported them to insert between the polymer chains better than other larger plasticizer molecules. On another hand, the elongation percentage increased in accordance with higher concentration of glycerol. The elongation percentage increased from 73.04±2.10% to 106.14±11.85% as the concentration of glycerol increased from 10% to 20% w/w, starch basis. Whereas decreasing glycerol concentration resulted in reduction of this property. Elongation relates to an ability of film to resist changes of shape before finally breaking due to plasticizers effect which weakened intermolecular hydrogen bonds between plasticizer and polymer molecules (Muscat et al., 2012).

Biodegradability of starch film

All starch-based films, including starch film mixed with 10, 15, 20% w/w plasticizer were buried in a soil at a depth of 10 cm. Each film showed different biodegradation periods, as presented in Figure 6.

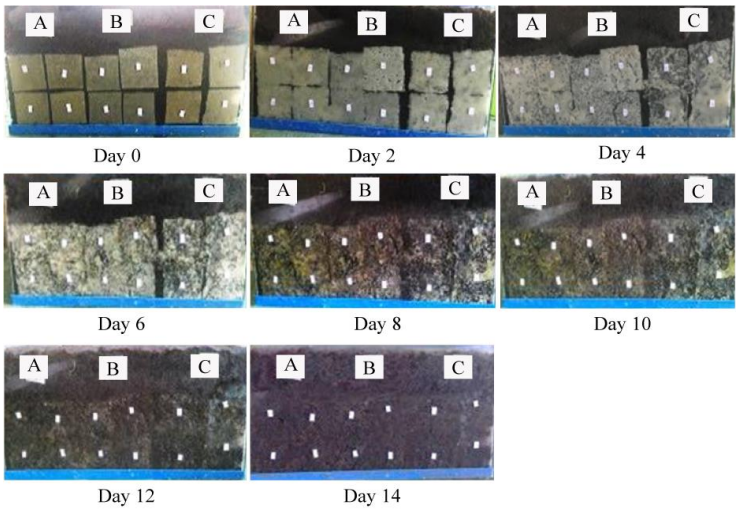


Figure 6 Biodegradability of glycerol blended starch film; (A): 10% glycerol blended starch film, (B): 15% glycerol blended starch film, (C): 20% glycerol blended starch film

The result showed that biodegradation of 20% glycerol-starch blend film started to be degrade on day 2 and completely degraded after 14 days (Figure 6). While the 10% and 15% glycerol-starch blend films were slower degraded than 20% glycerol starch film in the same condition. The plasticizer (glycerol) was a main

factor which defined the degradation rate of the blends. Glycerol had less mechanical resistance but higher strength than other plasticizers (Bourtoom, 2008). Thus, increasing in glycerol concentration enhanced the degradation rate of the film. There were some reports stating that glycerol, as plasticizer of rice starch-chitosan blend film, was more effective biodegradable material than polyethylene glycol (Bourtoom 2008; Obasi et al., 2013).

Antimicrobial activity of chitosan blended adlay starch film

The antibacterial activity of starch films blended with 0.5, 1.0, 1.5 and 2.0% w/w chitosan (Figure 7) were tested against *S. aureus* ATCC 25923, *E. coli* DMST 15537 and *P. aeruginosa* ATCC 27853, represent in Table 2. The inhibitory activity was evaluated based on measurement of the average diameter of clear inhibition zone.

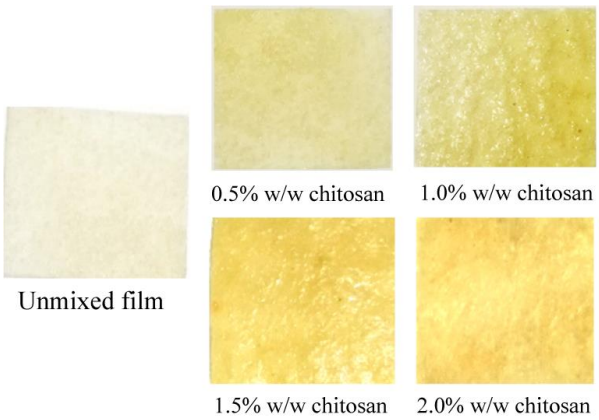


Figure 7 Chitosan-adlay starch blend film

Table 2 Antimicrobial activity of chitosan-adlay starch blend film against *S. aureus* ATCC 25923, *E. coli* DMST 15537 and *P. aeruginosa* ATCC 27853

Type of film	Inhibition zone diameter (mm)		
	S. aureus ATCC 25923	*E. coli* DMST 15537	*P. aeruginosa* ATCC 27853
Unmixed starch film	NZ	NZ	NZ
0.5% w/w chitosan film	NZ	NZ	NZ
1% w/w chitosan film	5.52±0.02	4.44±0.04	3.77±0.21
1.5% w/w chitosan film	6.00±0.00	5.90±0.02	6.00±0.00
2% w/w chitosan film	6.00±0.00	6.00±0.00	6.00±0.00
Gentamicin	22.33±0.58	21.00±0.00	21.33±0.58

NZ: No zone of inhibition presented

Agar plate test was performed to analyze inhibitory activity against three tested bacteria by examining the average diameter of clear inhibition zones. A starch film which was blended with four different chitosan component represented different size of clear inhibition zone. On the contrary, the films without chitosan (control) and the film blended with 0.5 w/w of chitosan showed no inhibitory effect against all tested bacteria. The greatest antimicrobial activity against *S. aureus* ATCC 25923 was observed for the starch films containing 1.5% and 2.0% chitosan. While the starch film which contained the highest chitosan concentration (2.0% w/w) exhibited the highest inhibitory activity on *E. coli* DMST 15537. For *P. aeruginosa* ATCC 27853, the starch films blended with 1.5% and 2.0% chitosan exhibited the largest zone of inhibition with a diameter of 6.0 ± 0.0 mm.

From the result, it was found that the efficiency of inhibition of bacterial growth depended on the concentration of chitosan. No inhibition zone was detected for a starch blended with low concentration of chitosan (0.5%w/w). Thus, an increasing of chitosan or bioactive compound in starch film may be a factor to enhance antimicrobial efficacy. It was also in agreement with the research of Chung and Chen (2008), Malinowska-Pańczyk et al., (2015) who reported that the highest antibacterial activity was observed for film blended with high chitosan component. Several researchers have shown that chitosan had antimicrobial potential against various bacteria (Malinowska-Pańczyk et al., 2015; Benhabiles et al., 2012; Elsabee and Abdou 2013). However, in this study, chitosan in film form showed very small inhibition effect. Generally, chitosan in aqueous solution exhibits more activities against gram-positive and gram-negative bacteria than chitosan in film form. The polymer chains of chitosan in solution are more flexible to interact either by hydrophilic or hydrophobic forces and it results in a greater antimicrobial activity than when it is in film form (Lim & Hudson, 2004). Chitosan could inhibit the growth of bacteria by changing and covering the outer membrane with vesicular structures on the cell surface of microbes. Therefore, the properties of the outer membrane of bacteria was disrupted (Toan et al., 2013). It showed more effective antimicrobial activity on gram-positive than gram-negative bacteria. Due to the component of outer cell wall of a gram-positive bacteria which was composed of teichoic acid and peptidoglycan with several pores, the structure facilitated the entry of foreign molecules or molecular adhesion. Whereas the cell wall of gram-negative bacteria contained lipopolysaccharide (LPS), proteins and internal peptidoglycan. Thus, this outer bilayer of membrane acted like a barrier against foreign molecules (Kong et al., 2010).

Effect of antibacterial films on shelf-life extension of fruit

Effect of antibacterial films on shelf-life extension of banana was observed for 14 days. The change in color was investigated and presented in Figure 8 and Table 3.

After 14 days storage, bananas which were packed into chitosan-starch blend films, can preserved for a longer period, compared to control. Among various treatments, the samples packed into starch films consisting of 1.5% and 2.0% w/w chitosan demonstrated increasing of shelf life to 14 days. For the maintenance of the skin color of bananas, the starch blended with 1.5% and 2.0% w/w chitosan showed

the color score as 4 (yellow with spots (1-20%)) and also showed a firm texture. On the contrary, an amount of brown pigments increased in the control treatment and in bananas coated with starch-chitosan (0.5% w/w) blend. Therefore, it was indicated that coating with the blend film of starch and high chitosan content encouraged the shelf life extension and reduced brown pigments in bananas.

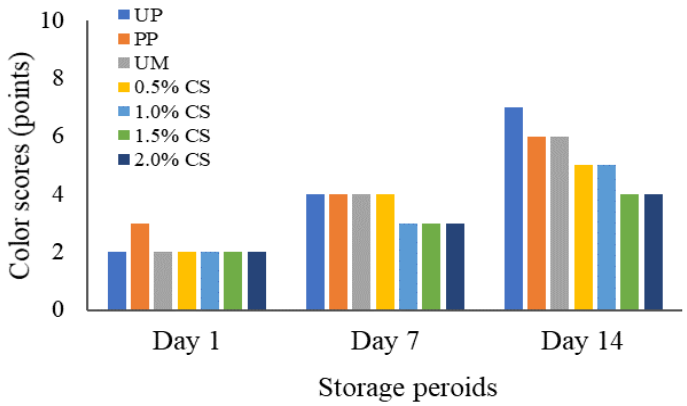
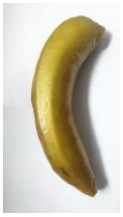


Figure 8 Color scores of bananas between the storage period (14 day)

Table 3 Effect of antibacterial films on shelf-life extension of bananas

Chitosan (% w/w)	Sample	Storage period (Days)			After storage period
		1	7	14	
unpacked					
petroleum plastic					
unmixed					

Table 3 Effect of antibacterial films on shelf-life extension of bananas

Chitosan (% w/w)	Sample	Storage period (Days)			After *storage period*
		1	7	14	
0.5%w/w					
1.0%w/w					 
1.5%w/w					 
2.0%w/w					 

Generally, banana is a typical climacteric fruit which produces ethylene during ripening process, resulting in moisture loss by the transpiration process (Golding et al. 1999). This can lead to peel color changes to brown, and finally black (Gane 1937). Bananas that were not coated with chitosan-starch blend film exhibited fast spoilage while coated bananas had prolonged shelf-life. The result agreed with those mentioned by Zhu et al. (2008) and Elsabee and Abdou (2013) and Mohamed et al. (2017) that chitosan had potential to be used as an antimicrobial agent for blending in biofilms or materials to promote extended the shelf-life. Chitosan could also reduce respiration rate of fruits even in a film form by controlling gas exchange though the fruit skin. It results in reducing of fruit metabolism, extends storage life and delay the ripening process (Elsabee and Abdou 2013; Aider 2010).

CONCLUSIONS

The results of this study indicated that the chitosan blended adlay starch film represented the efficient active packaging materials and promoted prolonged shelf life of fruits. The mechanical properties of films depended on the concentration of glycerol which was used as plasticizer to exhibit the flexible structure, however causing a decrease in tensile strength. In addition, increasing in glycerol concentration (20%w/w) facilitated the degradation rate of the film. The complete degradation of film was achieved within 14 days in the burial test. Furthermore, the greatest antimicrobial activity against three bacteria tested was performed by starch films containing 1.5% and 2.0% of chitosan concentration. The result indicated that bananas packed into starch films with 1.5% and 2.0% w/w chitosan enhanced their shelf life up to 14 days. Thus, the chitosan blended adlay starch films should be applied as active packaging materials to extend the shelf life of fresh fruits in the future. Moreover, it may be considered as a way for environmentally friendly packaging for fruits and other foods. However, more studies are still needed in order to develop better starch-based edible films for packaging in the further.

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