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ACTIVE BIOPACKAGING FROM DAMMAR FOR COMMINUTED MEAT PRESERVATION

[Pengemas Aktif dari Damar untuk Pengawetan Daging Cincang]

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ABSTRACT

This research aimed to develop biopackaging materials using thermoplastic starch matrix and Indonesian dammar extracts possessing antimicrobial activity, i.e. flesh dammar (*Shorea leprosula*) and stone dammar (*S. eximia*), for preserving comminuted meat. The packaging matrix was prepared using continuous melt mixing of tapioca starch and glycerol in a co-rotating twin extruder. Subsequently, the matrix was dipped in dammar extracts, with or without the addition of antimicrobial agents such as propyl paraben, zinc chloride, zinc acetate, and silver nitrate. As a result, flesh dammar performed greater antibacterial activity than that of stone dammar. Moreover, the antibacterial activity of silver nitrate in the biopackaging was comparable to that of zinc chloride while combined with dammar, but zinc acetate was less effective. On the other hand, active biopackaging comprised of combination of dammar and propyl paraben was the least effective. Among the eight combinations of two dammar extracts and four antimicrobial agents, flesh dammar extract comprised of 0.1% (w/v) zinc chloride and 1.0% (w/v) lecithin was found as the most promising formulation for dipping with regard to its production cost and antimicrobial activity. Total plate count (TPC) in comminuted meat wrapped with active biopackaging (initial microbial load of $5.2 \pm 0.1 \times 10^4$ CFU/g) decreased to $2.8 \pm 0.1 \times 10^4$ CFU/g over 9 days of storage at 40°C temperature. This number was lower than TPC value of nitrate-preserved meat ($3.4 \pm 0.2 \times 10^4$ CFU/g and $5.9 \pm 0.4 \times 10^5$ CFU/g, respectively).

Keywords: active biopackaging, comminuted meat, dammar, preservation

ABSTRAK

Penelitian ini bertujuan mengembangkan pengemas dari pati termoplastik sebagai matriks dan damar dari Indonesia yang mempunyai aktivitas antibakteri untuk mengawetkan daging cincang. Damar yang digunakan dalam penelitian ini adalah damar daging (Shorea leprosula) dan damar batu (S. eximia). Matriks disiapkan melalui pencampuran tepung tapioka dan gliserol dalam ekstruder ulir ganda putar searah. Kemudian, matriks dicelupkan ke dalam ekstrak damar, dengan dan tanpa penambahan propil paraben, seng klorida, seng asetat, dan perak nitrat sebagai antimikroba untuk kemasan. Hasil penelitian menunjukkan bahwa damar daging mempunyai aktivitas antibakteri yang lebih baik daripada damar batu. Aktivitas antibakteri dari perak nitrat setara dengan seng klorida bila dikombinasikan dengan damar, tetapi seng asetat kurang efektif. Disisi lain, pengemas aktif dari kombinasi damar dan propil paraben paling tidak efektif. Di antara delapan kombinasi dari dua spesies damar dan 4 agen antimikroba, formulasi terpilih untuk pencelupan adalah ekstrak damar daging yang mengandung 0,1% (b/v) seng klorida dan 1,0% (b/v) lesitin, berdasarkan biaya produksi dan aktivitas antimiroba dari pengemas aktif. Angka Lempeng Total (ALT) dalam daging cincang (mikroba awal $5,2\pm0,1 \times 10^4$ CFU/g) berkurang menjadi $2,8\pm0,1 \times 10^4$ CFU/g setelah dikemas dalam pengemas aktif dan disimpan selama 9 hari. Angka ini lebih rendah dari ALT pada daging yang diawetkan dengan nitrat ($3,4\pm0,2 \times 10^4$ CFU/g) dan pada daging dikemas dengan polietilen dan tidak diberi pengawet ($5,9\pm0,4 \times 10^5$ CFU/g).

Kata kunci: *daging cincang, damar, pengawetan, pengemas aktif*

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INTRODUCTION

Fresh meat is among the most nutrient-rich perishable food. Spoilage and pathogenic microbes favor to contaminate meat and cause a considerable lost to food processors. To prevent the microbial growth, various commercial preservatives such as nitrites and nitrates are commonly applied in processed meat. However, due to the toxicity issue, their concentration used in food products is confined by the regulation. Instead, Rhim and Ng (2007) reported that the use of antimicrobial packaging is more efficient than direct application of chemical preservative, by maintaining high concentration of antimicrobial agent on the food surface.

Nowadays, most people also prefer to consume "no preservative added" labeled foods. Therefore, the application of antimicrobial packaging is a smart strategy to preserve meat without possessing chemical hazard or deteriorating meat quality traits. Active packaging is an innovative concept that can be defined as a mode of packaging in which the package, product, and environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product (Suppakul *et al.,* 2003). Coating of films with antimicrobial agents can also result in effective antimicrobial activity (Kerry *et al.,* 2006). Both of them reported the mechanism of active packaging in inhibiting microbial growth, such as oxygen scavenging, moisture absorption and control, carbon dioxide and ethanol generation, and antimicrobial migrating and nonmigrating systems.

Assefa and Admassu (2013) reported that saponins extracted from haricot bean seeds revealed antimicrobial activity against *Escherichia coli, Salmonella typhi,* and *Enterobacter erogenous*. Our previous research also showed that some indigenous dammar from Indonesia possessed antibacterial activity, especially against Gram-positive bacteria (Mulyono *et al.,* 2012; 2013).

Another important issue is the environmental problem because of plastic packaging waste. The world seems to be wrapped in plastic. Almost every product, including meat, is encased in plastic. Plastic packaging waste becomes global issue because the disposal methods are still limited. Plastic recycling is considered inefficient because it produces complex multiphase products. Petroleum as raw material for plastic has also limited supply. Recently, biodegradable plastics made of renewable resources have been available to solve those problems. Some potential polymers for making biodegradable plastic are starch, chitosan, gluten, keratin, polylactic acid, and polyhydroxy butyrate, or the blends of them. However, the challenge to find out the ideal polymer is still open because the functional properties of current biodegradable plastics are still inferior and the processing cost is higher than that of conventional packaging plastics. Therefore, this research aimed to develop active biopackaging to extend the shelf life of fresh meat from renewable resources. The intended resources were tapioca starch, flesh dammar (*Shorea leprosula*) and stone dammar (*S. eximia*). All resources are abundant in Indonesia, the first one was used as the matrix and the other two were the antibacterial agents.

MATERIALS AND METHODS

Materials

Flesh dammar and stone dammar were purchased from CV Dammar Mustika Kencana Nusantara, a dammar exporter in Serpong and those dammar were collected from Malinau industrial forest in East Kalimantan on July 2009. Comminuted meat, polyethylene plastic, and tapioca starch were bought from a local market in South Jakarta. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Microbiology and Fermentation Laboratory, Faculty of Biotechnology, Atma Jaya Catholic University.

Matrix preparation

Thermoplastic starch (TPS) film was prepared by continuous melt mixing of tapioca starch and glycerol Merck (Taoyuan, Taiwan) at 130°C. The melt mixing was performed inside a laboratory scale corotating twin extruder (School of Chemical Engineering, Parahyangan Catholic University, Bandung, Indonesia) equipped with 3 cm screw diameter and 60 cm barrel length at screw rotation rate of 30 rpm. At the end of the extruder, a metal covering with a small gap (about 1.6 mm) in the center was installed to shape the resulting blend into a thin film of about 1.2 mm. Starch and glycerol were fed at total mass rate of 5.5 g/min, while the proportion of tapioca starch in the binary starch/glycerol blend is maintained at 70-75% (w/w).

Active biopackaging preparation

Flesh dammar and stone dammar were extracted with ethyl acetate separately Merck (Taoyuan, Taiwan), and each of them was filtrated to remove the insoluble matter. The soluble matter, which was biopolymer, was stored in amber reagent bottle for further use. Propyl paraben Merck (Taoyuan, Taiwan), zinc chloride Merck (Taoyuan, Taiwan), zinc acetate Merck (Taoyuan, Taiwan), and silver nitrate Merck (Taoyuan, Taiwan) were separately added to the biopolymer solution using

magnetic stirrer. To obtain homogenous dipping solution, lecithin Merck (Taoyuan, Taiwan) (with the weight of 10 times of the packaging additive) was added prior the antimicrobial incorporation. Afterwards, TPS was dipped in the biopolymer solution and dried in fume hood to obtain the active biopackaging. To obtain the optimum dosage of packaging additives, the optimization was applied in two steps; the first one at 0.2, 0.5, and 1.0%, and the second step depended on the result of the first step.

Antibacterial activity of active biopackaging against *S. aureus*

Active biopackaging was cut into a circle with diameter of 4 mm and placed onto Nutrient Agar (NA) Oxoid (Hampshire, United Kingdom) that had been inoculated by *S. aureus* and incubated at 37°C overnight. The appeared clear zone was then measured. Active biopackaging with the largest zone of inhibition from each additive was chosen for the next step.

Application of active biopackaging to preserve meat and its effectiveness compared to sodium nitrate

The comminuted meat (20 g weight) was wrapped by the chosen active biopackaging (treatment) or polyethylene (negative control). As positive control, sodium nitrate Merck (Taoyuan, Taiwan) solution was sprayed onto the comminuted meat, and left for 2 minutes to evaporate the water before packing in polyethylene (film thickness 0.03 mm) so that the concentration in meat reached 500 mg/kg. Subsequently, all packaged meat was stored at 4°C. The TPC in meat was daily analyzed for 9 days using pour plate method. Briefly, 1.5 g meat of each packaging treatment was put into 10 mL physiological saline solution and homogenized. Afterwards, it was diluted into 10^{-1}, 10^{-2} and 10^{-3}, and 1 mL of each solution was poured into the petridish and 15 mL of melted agar (45°C) was then added. After the agar had solidified, petri dish were inverted and incubated at 37°C for 24 hours.

Evaluation of the packaging additives migration into meat

The migration of packaging additives into meat over 9 days of storage was evaluated by extracting 1.5 g of packaged meat in 10 ml deionized water and the presence of silver or zinc in the supernatant was examined. The presence of silver was qualitatively shown by the formation of any precipitates if sodium chloride Merck (Taoyuan, Taiwan), sodium fluoride Merck (Taoyuan, Taiwan), potassium dichromate ($K_2Cr_2O_7$) Merck (Taoyuan, Taiwan), and sodium hydroxide Merck (Taoyuan, Taiwan) were added to supernatant (Svehla, 1997). The presence of zinc was quantitatively determined by titration the supernatant with ethylene diamine tetra acetate (EDTA) Merck (Taoyuan, Taiwan) standard solution (0.01 M) using Erichrome Black T Merck (Taoyuan, Taiwan) as indicator under basic condition (Jeffery *et al.,* 1989). Supernatant of the polyethylene-packaged meat was used as negative control.

Biodegradation of active biopackaging

The biodegradation of active biopackaging was evaluated using *P. aeruginosa* (Mulyono and Adrianus, 2012) for 4 weeks. The selected active packaging was immersed in Tryptose Soya Broth (TSB) Oxoid (Hampshire, United Kingdom) and incubated in shaking incubator at room temperature. On day 7, 14, 21, and 28 of incubation, the active packaging was withdrawn from media, rinsed, dried, and weighed. The biodegradation rate was defined as the percentage weight loss during incubation.

Statistical analysis

Triplicate experiments were performed throughout the study. All data were presented as the mean ± standard deviation (SD). The significance of differences between control and treated groups were statistically analyzed by Duncan Multi Range Test.

RESULT AND DISCUSSION

Antibacterial activity of active biopackaging against *S. aureus*

S. aureus was chosen in the study because it is known as common bacteria on human skin and nose and it has been recorded as one of many bacteria species that caused food borne disease (Bergdoll, 1989). The present study showed that TPS did not inhibit *S. aureus* growth, but after coated with dammar, it did. The inhibition zone of stone dammar-TPS and flesh dammar-TPS was 11 $\pm$ 1.2 and 13 $\pm$ 1.0 mm, respectively.

These findings were in accordance with our previous study, which reported the antibacterial activity of stone- and flesh-dammar extracts (Mulyono *et al.,* 2012; 2013). Using pyrolisis-GC/MS for chemical identification, Mulyono (2010[a]; 2010[b]) reported that the bioactive compounds in stone dammar and flesh dammar were similar, but their compositional percentages were different. The bioactive were sesquiterpene and sesquiterpene-O components, such as veridiflorol, α-copaene, δ-cadinene, and β-elemene. The presence of those compounds might play important role in their antibacterial activity. Cunico *et al.* (2007) reported that those compounds in the essential oil from

Ottonia martiana possessed antibacterial activities, especially against Gram-positive bacteria, including *S. aureus*. Another research reported that the hydroxyl group in sesquiterpene-O interferes the cell membrane stability (Solís *et al.,* 2004). Moreover, stone dammar has been reported as film forming material for transdermal application (Mundada and Avari, 2009). Table 1. presented the inhibition zone of active biopackaging containing combination of dammar extracts and packaging additives. The effect of silver nitrate and zinc chloride concentration to inhibition zone diameter was not linear when those additives were applied as packaging additive at 0.1-1.0%. Therefore, for the application step, concentration of those additives was set as 0.1% (1000 ppm).

Probably, the concentration of silver nitrate and zinc chloride used was higher than their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The MIC and MBC for silver on *S. aureus* were 120 and 160 ppm respectively (Ruparelia *et al.,* 2008), while the MIC for zinc chloride on *S. aureus* was 1000 ppm (Radhi, 2012). Dimitriu *et al.* (2006) also showed that there was correlation between MIC and diameter of inhibition zone. The lower the MIC, the greater the diameter of inhibition zone and vice versa. But if the concentration of antimicrobial agents were higher than their MIC/MBC values, the diameter of inhibition zone would depend on their diffusion rates. Compared to zinc acetate, zinc chloride was more effective as antimicrobial packaging. According to Babich and Stotzky (1978), the formation of various Zn-Cl species might exert greater antimicrobial activity than other divalent Zn^{2+}. The presence of zinc might induce morphological change, membrane leakage, and oxidative stress gene expression (Xie *et al.,* 2011). Besides, the exceptional data were shown by propylparaben. This chemical preservative has been widely used in food, cosmetic, and pharmaceutical. Bredin *et al.* (2005) reported that propylparaben induced potassium release in susceptible *E. coli* similar to that observed with polymyxin B (antibiotic derived from *Bacillus polymyxa*), and its efflux depended on porin channel activity. However, the result of the present study showed that the incorporation of propylparaben to dammar coating decrease the inhibition zone of the biopackaging (Table 1). This might be caused by the presence of lecithin According to Steinberg (2006), propylparaben could be inactivated by lecithin. Therefore, zinc acetate, zinc chloride, and silver nitrate, were chosen as packaging additives. The first one was used at 0.4% (w/v), while the other two at 0.1% (w/v).

The inhibition zone of active biopackaging containing stone dammar or flesh dammar extract was not as clear as that of active biopackaging containing the combination of dammar and packaging additives, especially combination of flesh dammar-silver nitrate and flesh dammar-zinc chloride. Clear area showed no bacteria growth and less clear area showed an inhibited growth. *S. aureus* might recover at a slower rate due to inferior inhibition by stone dammar or flesh dammar, but its growth was prevented by the addition of anti-microbial packaging agents (zinc chloride or silver nitrate). Therefore, it can be concluded that addition of zinc chloride or silver nitrate resulted in superior antimicrobial activities.

Application of active biopackaging to preserve meat and its effectiveness compared to sodium nitrate

The present study indicated that dammar possessed potent antibacterial activity compared to nitrate at dosage 500 mg/kg meat, wherein the flesh dammar showed better result than stone dammar did, although the difference was not significant (Table 2).

Table 1. Inhibition zone of active biopackaging containing damar over different packaging additive types and concentrations against *S. aureus*

Inhibition Zone (mm)								
	Flesh Dammar				Stone Dammar			
Additive Conc. (%w/v)	$AgNO_3$	Propyl Paraben	$ZnCl_2$	Zinc Acetate	$AgNO_3$	Propyl Paraben	$ZnCl_2$	Zinc Acetate
0.1	13.0 ± 1.2	NT	12.0 ± 1.0	NT	12.0 ± 1.5	NT	11.0 ± 2.1	0
0.2	13.0 ± 1.0	0	12.0 ± 1.2	1.0 ± 1.0	12.0 ± 1.1	0	12.0 ± 0.8	0
0.4	NT	NT	NT	11.0 ± 1.5	NT	NT	NT	11.0 ± 1.9
0.5	13.0 ± 0.6	0	11.0 ± 0.6	11.0 ± 1.0	12.0 ± 1.7	0	11.0 ± 1.5	11.0 ± 0.7
0.6	NT	NT	NT	11.0 ± 1.2	NT	NT	NT	11.0 ± 1.5
1.0	13.0 ± 0.5	3.0 ± 1.0	12.0 ± 0.5	8.0 ± 0.5	12.0 ± 0.5	1.8 ± 0.7	11.0 ± 0.1	7.0 ± 0.0

Note: NT = not tested

TPC in negative control (polyethylene-packaged meat) increased significantly during storage from day-0 to day-9. It indicated that microbes could grow in the polyethylene-packaged meat. Different results were showed by nitrate-preserved- and active biopackaging packaged-meats. In detail, TPC value at day-1 was less than at day-0, even though the two values were not statistically significant different and remain constant during 9 days of storage. It indicated that both nitrate and flesh dammar promoted antibacterial activity. Indonesian dammar, particularly flesh dammar, is thus potential to be developed as an active packaging material. Flesh dammar-starch based biopackaging expectantly can replace the current commercial meat packaging, which was 3-4 ply laminates comprised of ethyl vinyl acetate layer for outer protection, copolymer of vinylidene chloride for oxygen barrier, and irradiated ethyl vinyl acetate (Scanga, 2008). According to their TPC, all packaged meats over 9 days of storage at 4°C in the study were could be considered to be still safe for consumption because the TPC values were in the lower proposed range than the maximum TPC in beef permitted by FAO standard, which was 1.0 x 10^5 CFU/g (Heinz and Hautzinger, 2007). However, some countries may have different standards, for example Malaysia has more lenient regulation with maximum TPC of 10^6 CFU/g (Fazlina *et al.,* 2012) but India has more strict standard with maximum TPC of 10^3 CFU/g (FSSAI, 2011).

This study indicated that the presence of zinc or silver in the active biopackaging enhanced the antibacterial activity of active biopackaging (Table 3). The most effective antimicrobial agent for bio-packaging was the combination of flesh dammar with silver nitrate, followed by the combination of stone dammar with silver nitrate, and the combination of flesh dammar with zinc chloride, even though the difference was not significant. Moreover, the antimicrobial packaging was more effective to inhibit microbial growth than sodium nitrate at its maximum permitted level.

Table 2. Total plate count (TPC) in the comminuted meat packaged in active biopackaging containing dammar

	TPC (x10^4 CFU/g)			
Day	Negative Control	Positive Control	Flesh Dammar	Stone Dammar
1	2.2 ± 0.2^{b}	3.3 ± 0.2^{a}	4.3 ± 0.2^{a}	5.2 ± 0.1^{a}
2	3.0 ± 0.2^{c}	3.2 ± 0.1^{a}	4.1 ± 0.1^{a}	4.6 ± 0.2^{a}
3	3.2 ± 0.1^{cd}	3.2 ± 0.1^{a}	3.9 ± 0.1^{a}	4.4 ± 0.1^{a}
4	3.2 ± 0.0^{cd}	3.1 ± 0.1^{a}	3.7 ± 0.1^{a}	4.3 ± 0.0^{a}
5	3.3 ± 0.3^{d}	3.1 ± 0.1^{a}	3.8 ± 0.2^{a}	4.0 ± 0.1^{a}
6	3.7 ± 0.3^{e}	3.2 ± 0.3^{a}	3.8 ± 0.1^{a}	4.1 ± 0.1^{a}
7	4.1 ± 0.4^{f}	3.3 ± 0.1^{a}	3.9 ± 0.0^{a}	4.2 ± 0.1^{a}
8	4.9 ± 0.2^{g}	3.4 ± 0.1^{a}	3.9 ± 0.2^{a}	4.2 ± 0.1^{a}
9	5.9 ± 0.4^{h}	3.4 ± 0.2^{a}	4.0 ± 0.1^{a}	4.2 ± 0.2^{a}

Note: TPC in fresh comminuted meat at day-0 = (5.2±0.1) x 10^4CFU/g. Values followed by the same letter are not significantly different (P<0.05). Negative control: polyethylene, positive control: sodium nitrate

Table 3. Total plate count (TPC) (x10^4 CFU/g) in the comminuted meat packaged in active biopackaging containing the combination of dammar and packaging additives

	TPC (x10^4 CFU/g)					
Day	$AgNO_3$ + Flesh Dammar	$ZnCl_2$ + Flesh Dammar	ZnOAc + Flesh Dammar	$AgNO_3$ + Stone Dammar	$ZnCl_2$ + Stone Dammar	ZnOAc + Stone Dammar
1	2.8 ± 0.1	3.2 ± 0.1	3.6 ± 0.1	2.6 ± 0.4	3.3 ± 0.2	3.4 ± 0.2
2	2.6 ± 0.1	3.1 ± 0.1	3.3 ± 0.1	2.8 ± 0.1	3.1 ± 0.1	3.4 ± 0.1
3	2.5 ± 0.1	2.8 ± 0.1	3.2 ± 0.1	2.7 ± 0.1	3.0 ± 0.1	3.2 ± 0.1
4	2.3 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	3.0 ± 0.1
5	2.3 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	3.1 ± 0.1
6	2.4 ± 0.1	2.7 ± 0.2	3.1 ± 0.1	2.7 ± 0.2	3.0 ± 0.1	3.1 ± 0.2
7	2.3 ± 0.1	2.7 ± 0.1	3.0 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	3.1 ± 0.1
8	2.4 ± 0.1	2.8 ± 0.1	3.1 ± 0.1	2.7 ± 0.2	3.0 ± 0.1	3.1 ± 0.3
9	2.5 ± 0.1	2.8 ± 0.1	3.2 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	3.2 ± 0.1

Note: TPC in fresh comminuted meat at day-0 = (5.2±0.1) x 10^4 CFU/g

According Vaisanen *et al.* (1991), microbial contamination in packaged food did not only come from the food inside, but also from the bacteria contamination on food packaging, especially the aerobic strains and sporeformers. In addition, chemical preservative, especially nitrites and nitrates, are not safe. Nitrates have been suspected of playing a role in brain cancer, childhood leukemia, and reproductive toxicity (Sindelar and Milkowski, 2012).

Packaging additives migration into meat

The migration test showed that the concentration of zinc in meat increased about 20 and 33 ppm for the meat wrapped by active biopackaging containing zinc chloride (0.1% w/v) and zinc acetate (0.4% w/v), respectively. However, the meat itself contained approximately 10-25 ppm zinc, and the US National Institute of Health (NIH) tolerable upper intake level intake of zinc for adult men is 40 mg/day (NIH, 2011). It thus indicated that the use of antimicrobial packaging containing zinc chloride (0.1%) for meat did not give any adverse health effect. There were no precipitate formed in the meat broth over addition of sodium chloride, sodium fluoride, potassium dichromate, and sodium hydroxide solutions. According to the silver nitrate solubility in water, it could be estimated that the concentration of silver in the meat packaged in active biopackaging containing silver nitrate was less than 1.45 ppm.

The acceptable daily intake of silver was 350 µg/day (EC, 2000); accordingly, the maximum daily consumption of packaged meat comprising silver is about 200 g. According the price of the raw material of coating available online, it can be roughly predicted that the additional cost for producing antimicrobial packaging was USD 4.30-8.71/m^2 coating (Table 4). Zinc chloride can thus be proposed as the most promising antimicrobial agent for flesh dammar-starch based packaging.

Table 4. Additional cost needed for producing antimicrobial damar biopackaging

Material for Dipping	Cost (USD/m^2 film)
Flesh dammar	4.30
Flesh dammar + 0.1% $AgNO_3$	8.71
Flesh dammar + 0.1% $ZnCl_2$	4.39
Flesh dammar + 0.4% ZnOAc	4.66

All active biopackaging could be biodegraded using *P. aeruginosa*, but the rate depended on the composition of packaging (Figure 1). The degradation pattern of the uncoated TPS which had less antimicrobial activity and lack of a lag phase demonstrated its superior biodegradability. On the other hand, the degradation pattern of active packaging containing flesh dammar, with or without the addition of silver and zinc ions was similar. In the initial stage, biodegradation rate was slow, but after two weeks of treatment, it increased (Table 5). This study presented that thermoplastic starch could be completed degraded in 6 weeks, while the active biopackaging needed 7-10 weeks for biodegradation. Active biopackaging comprised of flesh dammar extract required 7 weeks, but the presence of zinc as zinc chloride or zinc acetate in active biopackaging slowed down the biodegradation to about 8 weeks. Active biopackaging comprised of flesh dammar extract and silver nitrate required the longest time to be fully degraded (almost 10 weeks).

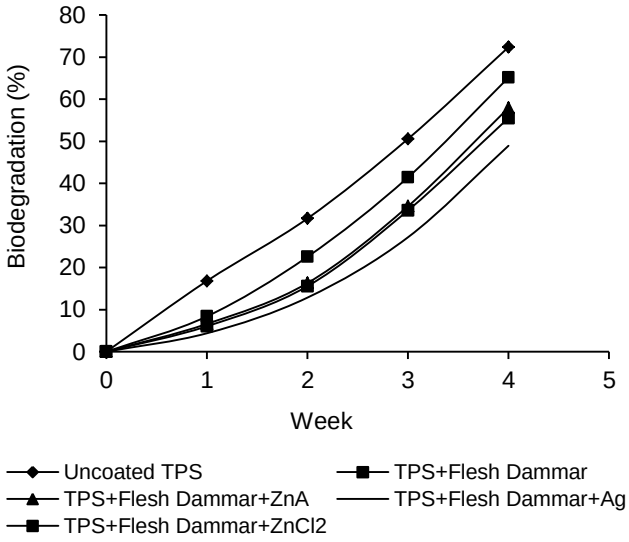

Figure 1. Biodegradation pattern of active biopackaging using *P. aeruginosa* as test bacteria

Table 5. Biodegradation rate of TPS and active packaging

Composition	Initial Rate (%/Week)	Normal Rate (%/Week)
TPS	17.85	17.85
TPS + flesh dammar	11.27	15.71
TPS + flesh dammar + $ZnCl_2$	7.78	13.26
TPS + flesh dammar + ZnOAc	8.15	13.80
TPS + flesh dammar + $AgNO_3$	6.42	11.50

CONCLUSION

Flesh dammar could be developed as coating for active biopackaging. Its effectiveness in inhibiting the microbial growth in comminuted meat is

comparable to direct preservation using sodium nitrate. Furthermore, its effectiveness could be increased by the addition of antimicrobial agents such as silver nitrate and zinc chloride. It is also compatible to be applied with thermoplastic starch. The active biopackaging is also biodegradable at the rate of 11.50-15.71% (w/w) after passing their lag phase for the first 2-weeks. Further research about the effect of active biopackaging to other aspect of meat quality such as texture, taste, and water binding capacity should be investigated.

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