International Journal of Food Microbiology 290 (2019) 59–67

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Thermoplastic starch/polybutylene adipate terephthalate film coated with gelatin containing nisin Z and lauric arginate for control of foodborne pathogens associated with chilled and frozen seafood

Rinrada Pattanayaiying[a,*], Amporn Sane[b], Penchom Photjanataree[c], Catherine N. Cutter[d]

[a] Department of Industrial Microbiology, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok 10300, Thailand
[b] Department of Packaging and Material Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand
[c] Thai Packaging Centre, Thailand Institute of Scientific and Technology, 196 Phahonyothin Rd., Chatuchak, Bangkok 10900, Thailand
[d] Department of Food Science, College of Agricultural Sciences, 433 Rodney A. Erickson, Food Science Building, The Pennsylvania State University, University Park, PA 16802, USA

ARTICLE INFO

Keywords:
Antimicrobial edible film
Vibrio parahaemolyticus
Salmonella
Bigeye snapper
Tiger prawn

ABSTRACT

In order to control foodborne pathogens on seafood products, an antimicrobial, thermoplastic starch/polybutylene adipate terephthalate (TPS/PBAT; 40/60) film was produced by coating gelatin (15% v/v) containing lauric arginate (LAE; 0.8 mg/cm^2), alone or combination with nisin Z (69.4 AU/cm^2) to produce LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films, respectively. Both films were investigated for control of *Vibrio parahaemolyticus* ATCC 17802 and *Salmonella* Typhimurium ATCC 14028 on bigeye snapper (*Lutjanus lineolatus*) and tiger prawn (*Penaeus monodon*) slices during long-term (28 days), refrigerated (4 °C; chilled) and frozen (−20 °C) storage up to 90 days. *S.* Typhimurium ATCC 14028, experimentally inoculated onto bigeye snapper and tiger prawn slices, treated with the LAE-Gelatin-TPS/PBAT film, and stored at 4 °C was reduced 3.2 $\log_{10}$ CFU/g after 28 days and 7 $\log_{10}$ CFU/g after 21 days, respectively. Nisin-LAE-Gelatin-TPS/PBAT film reduced *S.* Typhimurium ATCC 14028 on bigeye snapper and tiger prawn slices 3.5 $\log_{10}$ CFU/g after 28 days and 7 $\log_{10}$ CFU/g after 14 days at 4 °C, respectively. The LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films and storage for 28 days at 4 °C reduced *V. parahaemolyticus* inoculated on chilled bigeye snapper slices approximately 2.6 and 4.2 $\log_{10}$ CFU/g, respectively. Both films reduced *V. parahaemolyticus* inoculated on chilled tiger prawn slices approximately 7.1 $\log_{10}$ CFU/g after 28 days at 4 °C. The LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films also reduced *S.* Typhimurium, inoculated on bigeye snapper and tiger prawn slices, 5.8 and 5.6 $\log_{10}$ CFU/g, respectively, after 60 days at −20 °C. *V. parahaemolyticus* was reduced by 5.8 $\log_{10}$ CFU/g on frozen bigeye snapper and tiger prawn slices after treatment with Nisin-LAE-Gelatin-TPS/PBAT film after 14 and 21 days, respectively. However, the LAE-Gelatin-TPS/PBAT film reduced *V. parahaemolyticus* 5.8 $\log_{10}$ CFU/g on both frozen seafood slices after 28 days. The results obtained from this study indicate the LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films displayed excellent inhibition against *S.* Typhimurium and *V. parahaemolyticus* on chilled and frozen seafood.

1. Introduction

Seafood has long been an important part of a healthy diet. Consumption of seafood is associated with potential health benefits, including neurologic development during gestation and infancy (Hibbeln et al., 2007; Iwamoto et al., 2010) and a reduced risk of heart disease (Iwamoto et al., 2010; Kris-Etherton et al., 2002). However, seafood also may be responsible for worldwide foodborne illnesses and outbreaks. In the United States (U. S.), the Centers for Disease Control and Prevention has reported that fish and shellfish account for 10% of all foodborne illness outbreaks, with most of the outbreaks resulting from the consumption of raw molluscan shellfish (Olgunoglu, 2012).

Seafood may be a vehicle for bacterial pathogens, such as *Vibrio* and *Salmonella* spp. (Bakr et al., 2011; Huss, 1997; Letchumanan et al., 2014). *V. parahaemolyticus* was first discovered by Tsunesaburo Fugino in 1950 as a causative agent of foodborne disease following a large outbreak in Japan resulting in 272 illnesses, with 20 deaths (Letchumanan et al., 2014). Since *V. parahaemolyticus* occurs naturally

* Corresponding author.
E-mail address: rinrada.pa@ssru.ac.th (R. Pattanayaiying).

https://doi.org/10.1016/j.ijfoodmicro.2018.09.015
Received 24 April 2018; Received in revised form 2 September 2018; Accepted 16 September 2018
Available online 18 September 2018

in marine environments and is a cause of gastroenteritis linked to seafood consumption in the U. S. (Iwamoto et al., 2010), it has been a public health concern, causing import bans, detention and rejections associated with the international fish trade (Bakr et al., 2011; Letchumanan et al., 2014; Nelapati et al., 2012; WHO, 2001). Meanwhile, *Salmonella* spp. may contaminate seafood during production or processing, and cross-contaminate products during various stage of preparation (Amagliani et al., 2012). The presence of *Salmonella* in seafood has been reported in India (Kumar et al., 2003; Shabarinath et al., 2007), China (Yang et al., 2015), Singapore (Huang et al., 2012), Sri Lanka (Fonseka, 1990) and Thailand (Rattagol et al., 1990). The U.S. Food and Drug Administration (FDA) has demonstrated that *Salmonella* spp. was the most common contaminant of fish and fishery products, with approximately 58% of *Salmonella* spp. contamination in seafood linked to shrimp and prawns (Allshouse et al., 2004). Therefore, consumption of raw or undercooked seafood is recognized as a health risk to consumers.

Since *V. parahaemolyticus* and *Salmonella* spp. are able to survive under freezing conditions for long periods of time (Amagliani et al., 2012; Vasudevan et al., 2002), the contamination of these bacteria in chilled and frozen seafood products is a serious problem for the seafood industry. Control of these pathogens with certain chemicals and/or certain packaging materials has raised consumer concerns, since they are believed to be hazardous to human health and to the environment. However, packaging made from natural compounds, along with the incorporation of food grade antimicrobials, could be more effective for controlling the pathogens in seafood, while decreasing consumer concerns.

Nisin and lauric arginate (LAE) are safe and effective antimicrobials for food applications. Nisin Z is a small antimicrobial peptide produced by *Lactococcus lactis* subsp. *lactis* I8-7-3 LC113942 (Pattanayaiying et al., 2015). Nisin has been approved by the FDA as Generally Recognized as Safe (GRAS) via Notice No. GRN000065 (FDA, 2001). However, the limitation of nisin as an antimicrobial is its narrow spectrum of inhibitory activity. It is active against Gram-positive bacteria, with limited activity against Gram-negative bacteria. Lauric arginate (Nα-lauryl-L-arginine ethyl ester monohydrochloride; LAE) is a derivative of lauric acid, L-arginine, and ethanol. LAE also has been recognized as GRAS by the FDA for use as an antimicrobial at levels up to 225 mg/kg in specific food categories (FDA, 2005). LAE is a broad-spectrum antimicrobial that causes disruption or instability of the plasma membrane lipid bilayer, alters metabolic processes, and hinders the cellular cycle without cellular lysis (Bakal and Diaz, 2005; Pattanayaiying et al., 2015; The Target Group, Inc., 2012). Finally, the combination of LAE and nisin Z enhances the antimicrobial activity against Gram-negative bacteria, including *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Salmonella* Typhimurium (Pattanayaiying et al., 2014; Pattanayaiying et al., 2015).

Many reports have demonstrated that edible films incorporated with antimicrobials, including essential oils, LAE and bacteriocins, could be effective for reduction of foodborne pathogens. Emiroglu et al. (2010) indicated that a soy protein film incorporated with thyme and oregano essential oil significantly inhibited *E. coli* and *Staphylococcus aureus*. Trinetta et al. (2010) demonstrated that sakacin A-containing pullulan film could effectively reduce *Listeria monocytogenes* on turkey breast slices. Pattanayaiying et al. (2015) illustrated that pullulan film incorporated with LAE and nisin Z inhibited foodborne pathogens on fresh and ready-to-eat muscle foods. Kashiri et al. (2016) demonstrated that LAE could be released from active zein films and inhibit *L. monocytogenes* and *E. coli*. However, the commercial use of the antimicrobial edible films has been limited because of their poor mechanical properties.

Thermoplastic starch/polybutylene adipate-*co*-terephthalate (TPS/PBAT) films are made from a blend of PBAT with cassava starch (Brandelero et al., 2012). The resulting film is a biodegradable polymer with good mechanical properties, including transparency, strength, and elasticity. It is possible that this film may provide an alternative to existing packaging materials and be combined with food-grade antimicrobials to control pathogens associated with seafood products. Therefore, the objective of this research was to investigate whether a TPS/PBAT film coated with gelatin containing LAE and nisin Z, alone or in combination, could control foodborne pathogens on chilled and frozen seafood during long term storage.

2. Material and methods

2.1. Bacterial strains and culture conditions

Lactococcus lactis subsp. *lactis* I8-7-3 LC 113942, the bacteriocin-producing lactic acid bacterium, was grown and produced nisin Z in modified *Lactobacillus* de Man Rogosa, Sharpe (MRS) broth (BD, Franklin Lakes, NJ, USA), with the addition of dextrose (5%; Ajax Finechem Pty Ltd, Auckland, New Zealand) at 30 °C and under anaerobic conditions (Anaerocult; Merck, Darmstadt, Germany).

E. coli O157:H7 ATCC 43895, *Salmonella* Typhimurium ATCC 14028, *Salmonella* Enteritidis ATCC 10118, *S. aureus* ATCC 12600 and *V. parahaemolyticus* ATCC 17802 were purchased from American Type Culture Collection (ATCC, Arlington, Virginia, USA). *L. monocytogenes* Scott A was obtained from the Penn State Food Microbiology Culture Collection. All bacterial strains were grown in Tryptic Soy Broth (TSB; BD) containing 0.6% of yeast extract (BD) at 37 °C (Pattanayaiying et al., 2015).

The selective media for enumeration of *S.* Typhimurium, and *V. parahaemolyticus* from foods were *Salmonella*-Shigella agar (SS; BD) and Thiosulfate Citrate Bile Salts Sucrose agar (TCBS; BD), respectively.

2.2. Preparation of partially purified nisin Z

L. lactis subsp. *lactis* I8-7-3 LC 113942, a nisin *Z*-producing lactic acid bacterium, was isolated from the gut of walking catfish (Pattanayaiying et al., 2012). Cultivation of *L. lactis* subsp. *lactis* I8-7-3 LC 113942, production and partial purification of nisin Z were performed as described previously (Pattanayaiying, 2015). Briefly, *L. lactis* subsp. *lactis* I8-7-3 LC 113942 was grown in MRS broth at 30 °C under anaerobic conditions for 24 h. The supernatant was harvested by centrifugation at 8000 rpm for 15 min at 4 °C and subsequently heated at 80 °C for 20 min to destroy proteolytic enzymes and any living cells. The cell-free-supernatant was mixed with 12% w/v of amberlite XAD-16 resin (Sigma-Aldrich; Steinheim, Germany) and gently shaken (60 rpm) at room temperature for 3 h. The absorbed nisin Z was eluted from amberlite XAD-16 resin with 70% isopropanol (Fisher Scientific; Leices, UK), pH 2. The eluent was adjusted to pH 5.5 and isopropanol was removed by evaporation at 60 hPa, 42 °C (Pattanayaiying, 2015). The partially purified nisin Z was kept at −20 °C before coating on the films.

2.3. Preparation of active TPS/PBAT films

2.3.1. Preparation of thermoplastic resin from cassava starch and LAE by extrusion process

The thermoplastic resin was prepared by mixing cassava starch with glycerol (plasticizer) in a 20-l mixer (Mitsubishi, Japan) at the speed level 2, for 30 min. The ratio of cassava starch: glycerol was fixed at 100:40. LAE was also added to obtain a final concentration of 0, 2.45, 3.64, 4.81 and 5.96% respectively. The mixtures were subsequently extruded using a co-rotating intermeshing twin-screw extruder (LTE-20-40, Labtech Engineering, Thailand) with a ratio of length and diameter (L/D) at 40:1, 20 mm diameter of screw and 3 mm diameter of die. The extruded plastic was cut into 2.5 mm length of thermoplastic resin by using LZ-120 Labtech scientific pelletizer (Labtech Engineering) at a speed of 25 m/min. The resin was dried in a hot air oven at 50 °C and kept at room temperature in a closed container

Table 1
The composition of thermoplastic starch (TPS) or lauric arginate-thermoplastic starch (LAE-TPS) resins and polybutylene adipate terephthalate (PBAT) resin for preparation of thermoplastic starch/polybutylene adipate terephthalate (TPS/PBAT) or lauric arginate-thermoplastic starch/polybutylene adipate terephthalate (LAE-TPS/PBAT) blended resins.

Blended Resin	Ingredient of resin		Total (g)
	TPS or LAE-TPS resin (g)	PBAT resin (g)	
TPS/PBAT	600.0	900.0	1500.0
1.0% LAE-TPS/PBAT	612.0	888.0	1500.0
1.5% LAE-TPS/PBAT	618.0	882.0	1500.0
2.0% LAE-TPS/PBAT	624.0	876.0	1500.0
2.5% LAE-TPS/PBAT	629.0	871.0	1500.0

containing silica gel until the film formation was performed.

2.3.2. Preparation of TPS/PBAT or LAE-TPS/PBAT blended resins by extrusion process

The (LAE-)TPS and PBAT resins were melted and blended together to form TPS/PBAT or LAE-TPS/PBAT resins by using a twin-screw extruder (LTE-20-40, Labtech Engineering, Thailand). The composition of blended resin is shown in Table 1.

2.3.3. Formation of the TPS/PBAT and LAE-TPS/PBAT blended films by blown film extrusion process

The TPS/PBAT or LAE-TPS/PBAT resins were used to prepare film by using single screw extrusion blow molding (LE 25–30/C, Labtech Engineering) with a 25 mm screw diameter and a 30:1 L/D ratio. Film thickness was in the range of 30–35 μm. The films were kept at room temperature in a closed container until the experiment or analysis was performed.

2.3.4. Direct coating with antimicrobial on the TPS/PBAT film

LAE (CytoGuard Lauric Arginate, A&B Ingredient; NY, USA) was prepared and directly coated on the TPS/PBAT film (0.0–0.16 mg/cm^2) and allowed to dry under a laminar hood for 24 h at room temperature (25 ± 2 °C) and 35% RH. The dry film was stored at room temperature (25 ± 2 °C) and 35% RH. The film was determined for antimicrobial activity against foodborne pathogens by a plate overlay assay as described in Section 2.4.

2.3.5. Coating of the antimicrobial(s)-containing polymer onto the TPS/PBAT film

2.3.5.1. Coating of LAE-containing polymer onto the TPS/PBAT film. Since direct coating and incorporation of LAE by the blown film extrusion process did not allow for the release of LAE from TPS/PBAT film (data not presented), gelatin or pullulan were considered for improvement of the antimicrobial release. Therefore, a bilayer film was developed by coating the LAE-containing polymer onto the TPS/PBAT film. Primarily, gelatin (Merck Chemical; NH, UK) was dissolved by stirring continuously in distilled water to obtain a final concentration of 12 or 15% v/v, respectively. Ten milliliters of the polymer solution, containing 1.6 mg/ml of LAE, were coated onto 10 × 10 cm (100 cm^2) pieces of TPS/PBAT film to obtain 0.16 mg LAE/cm^2 and dried under a laminar hood for 24 h at 25 ± 2 °C and 35% RH. The dried film was stored at room temperature (25 ± 2 °C) and 35% RH until the antimicrobial activity of the film was determined.

2.3.5.2. Coating of LAE-Nisin Z containing polymer onto the TPS/PBAT film. Gelatin solutions with antimicrobials were prepared for coating onto the TPS/PBAT film by addition of LAE alone or combination with nisin Z to obtain a final concentration on the TPS/PBAT film of 0.16 mg/cm^2 and 69.4 AU/cm^2, respectively. The active bilayer films were dried under a laminar hood for 24 h at 25 ± 2 °C and 35% RH. Gelatin solutions without antimicrobials also were coated on the TPS/PBAT films, dried as described above, and used as controls (gelatin-TPS/PBAT) film. The dry films were stored at room temperature (25 ± 2 °C) and 35% RH until experiments were performed.

2.4. Determination of antibacterial activity of the active TPS/PBAT films

The antibacterial activity of the active TPS/PBAT films was determined by a plate overlay assay (Pattanayaiying, 2015). The active TPS/PBAT films were aseptically cut into pieces (1 cm × 1 cm). Each piece of film was placed onto a layer of TSB containing 0.6% yeast extract and 0.75% agar (BD) seeded with 6 $\log_{10}$ CFU/ml of overnight cultures *E. coli* O157:H7 ATCC 43895, *L. monocytogenes* Scott A, *S. aureus* ATCC 12600, *S.* Typhimurium ATCC 14028, *S.* Enteritidis ATCC 10118 or *V. parahaemolyticus* ATCC 17802 and incubated for 24 h at 37 °C (Pattanayaiying, 2015). The plates were subsequently observed for inhibition zones against the indicator lawn caused by the antimicrobial films. The TPS/PBAT film without the antimicrobial agent also was used as a negative control.

2.5. Inoculation of seafood

Bigeye snapper (*Lutjanus lutjanus*) and tiger prawn (*Penaeus monodon*) were purchased from a local supermarket and transported at 4 °C to the laboratory. The bigeye snapper was aseptically cut into slices (5 cm × 5 cm). Meanwhile, the tiger prawn was sectioned longitudinally, cut and weighed to 10 g. All seafood slices were exposed to ultraviolet light (emission of 253.7 nm.) in a biological safety hood (Biological Safety Cabinet 11239BBC86, Biobase; Jinan, China) for 15 min/side to reduce populations of indigenous bacteria on the samples (Cutter and Siragusa, 1998).

Inoculum of *S.* Typhimurium ATCC 14028 or *V. parahaemolyticus* ATCC 17802 was prepared by cultivation of each bacterial strain in TSB containing 0.6% of yeast extract at 37 °C for an overnight. The inoculums were diluted serially 1:10 in sterile buffered peptone water (BPW; BD) or alkaline peptone water (APW; BD) for enumeration of *S.* Typhimurium ATCC 14028 or *V. parahaemolyticus* ATCC 17802, respectively. The diluted inoculums were made into a cocktail (1:1) and 1 ml of the cocktail mixture was spread onto one side of the surface-treated raw bigeye snapper or tiger prawn slices to reach a final concentration of approximately 6 $\log_{10}$ CFU/g. The inoculated samples were incubated statically in the biological safety hood at room temperature for 20 min to allow for bacterial attachment (Pattanayaiying et al., 2015). All media for cultivation or enumeration of *V. parahaemolyticus* ATCC 17802 were supplemented with 3% sodium chloride (NaCl).

2.6. Packaging of inoculated seafood samples

The inoculated seafood samples were treated with different gelatin-coated TPS/PBAT films as follows: 1) the gelatin-coated TPS/PBAT films without antimicrobial agents (control film; Gelatin-TPS/PBAT); 2) the TPS/PBAT films coated with gelatin containing LAE (LAE-Gelatin-TPS/PBAT); and 3) the TPS/PBAT films coated with gelatin containing LAE and nisin Z (Nisin-LAE-Gelatin-TPS/PBAT). Additionally, the inoculated seafood samples without film were used as negative controls in this experiment.

Inoculated, either treated or untreated samples were transferred individually into a standard vacuum-packaging bag (Foil Packaging; Chonburi, Thailand) and subsequently vacuum-packed using a DZ-260/PD Table Vacuum Packager (Wenzhou Huaqiao Packing Machine Factory; Wenzhou, China). The vacuum-packed samples were stored at either 4 °C for up to 28 days or −20 °C for up to 2 months, until sampled.

Table 2
Antibacterial activity of thermoplastic starch/polybutylene adipate terephthalate (TPS/PBAT) films incorporated or directly coated with lauric arginate (LAE) against foodborne pathogens in a plate overlay assay.

	Inhibition zone (mm^2) of TPS\PBAT films incorporated with LAE (mg/cm^2)					Inhibition zone (mm^2) of TPS/PBAT directly coated with LAE (mg/cm^2)[1]				
Bacterial indicators	0.00	0.02	0.04	0.08	0.16	0.00	0.02	0.04	0.08	0.16
S. Typhimurium ATCC 14028	0	0	0	0	0	0$^{(a)}$	0$^{(a)}$	144.00 ± 9.8$^{(b)}$	402.00 ± 9.64$^{(c)}$	633.00 ± 12.01$^{(d)}$
S. Enteritidis ATCC 10118	0	0	0	0	0	0$^{(a)}$	0$^{(a)}$	203.00 ± 4.97$^{(b)}$	399.00 ± 11.53$^{(c)}$	599.67 ± 7.77$^{(d)}$
L. monocytogenes Scott A	0	0	0	0	0	0$^{(a)}$	0$^{(a)}$	243.00 ± 8.04$^{(b)}$	599.33 ± 11.71$^{(c)}$	825.00 ± 10.54$^{(d)}$
S. aureus ATCC 12600	0	0	0	0	0	0$^{(a)}$	0$^{(a)}$	114.33 ± 5.43$^{(b)}$	199.67 ± 7.63$^{(c)}$	238.33 ± 15.31$^{(d)}$
E. coli O157:H7 ATCC 43895	0	0	0	0	0	0$^{(a)}$	110.67 ± 10.5$^{(b)}$	158.33 ± 6.23$^{(c)}$	404.33 ± 8.62$^{(d)}$	669.00 ± 12.76$^{(e)}$
V. parahaemolyticus ATCC 17802	0	0	0	0	0	0$^{(a)}$	108.3 ± 3.79$^{(b)}$	225.00 ± 8.98$^{(c)}$	401.00 ± 9.85$^{(d)}$	746.67 ± 7.64$^{(e)}$

[1] Values in the same row followed by different letters significantly differed ($P < 0.05$).

2.7. Microbial enumeration of the foodborne pathogens in the treated samples

The samples were determined for remaining pathogen populations after storage at 4 °C for 0, 3, 7, 14, 21, and 28 days or −20 °C for 0, 3, 7, 14, 21, 28 and 60 days. Each treatment was analyzed in triplicate. Frozen samples were thawed at 4 °C for 8 h. After aseptic opening of packages, samples were transferred aseptically to a stomacher bag (Seward; West Sussex, UK) and stomached for 2 min in BPW or APW for enumeration of *S.* Typhimurium ATCC 14028 or *V. parahaemolyticus* ATCC 17802, respectively. Ten-fold serial dilutions of the samples were prepared and spread onto SS agar (BD) or TCBS agar (BD) plates for enumeration of *S.* Typhimurium ATCC 14028 or *V. parahaemolyticus* ATCC 17802, respectively. All plate counts were performed in duplicate and the plates were incubated at 37 °C for 24 h. Bacterial colonies were counted manually, converted, and expressed as $\log_{10}$ CFU/g.

2.8. Statistical analysis

All challenge experiments were performed in triplicate and the selected serial dilution was plated in duplicate. The bacterial counts from duplicate plates were averaged and transformed to $\log_{10}$ CFU/g (Trinetta et al., 2010). All statistical analyses were performed with SPSS Software (IBM, SPSS Statistics 20 free download, Pennsylvania State University, University Park, PA). Significant differences were evaluated with general linear model followed by Tukey multiple comparison test. The values were defined as statistical significance at $P < 0.05$.

3. Results and discussion

3.1. Antibacterial activity of active TPS/PBAT films

The antimicrobials were incorporated into TPS/PBAT film by different methods, including direct incorporation, direct coating, or a coating by combination with gelatin solutions. The incorporation of LAE into TPS/PBAT resin by co-extrusion did not demonstrate any antibacterial activity against all test strains of the experiment (Table 2). Cuadrado et al. (2016) have demonstrated similar results using the incorporation of zinc oxide, zinc acetate, and potassium sorbate into low-density polyethylene (LDPE). Co-extrusion offered poor results and low antimicrobial activity. Given this information, we believe that the antimicrobials cannot be released or are released very slowly from the polymer into the inoculated medium.

Since the film produced by direct incorporation of LAE to the TPS/PBAT resin by co-extrusion was not active against any bacterial strains, the TPS/PBAT film was coated with LAE. Direct coating of LAE on TPS/PBAT film varied in final LAE concentration to between 0 and 0.16 mg/cm^2. The TPS/PBAT film directly coated with 0.16 mg/cm^2 of LAE displayed the largest inhibition zone against all of the test strains; therefore, other coating methods including incorporation of LAE into pullulan or a gelatin polymer were performed at 0.16 mg/cm^2 of LAE. Since TPS/PBAT film coating with LAE in gelatin polymer displayed higher antimicrobial activity against all indicator strains than the TPS/PBAT film coating with LAE in pullulan polymer (Table 3), gelatin was chosen as the polymer for coating of antimicrobials with either LAE alone or LAE in combination with nisin Z for further experiments. Min et al. (2010) reported that 0.5% Nisaplin-incorporated gelatin film was effective in inhibiting *L. monocytogenes* on bologna during storage at 4 °C for 56 days. The use of antimicrobial additives in gelatin-based films or coatings for food packaging application is a promising area, with the main goal being the prolongation of food shelf life based on retarding deterioration mechanisms inside the package by using natural additives (Ramos et al., 2016). Furthermore, LAE was used at a lower concentration, 0.08 mg/cm^2, with a combination of nisin Z, incorporated with gelatin and coated on the TPS/PBAT film in the present study.

The combination of LAE (0.08 mg/cm^2) with Nisin Z, which was varied in concentration between 0 and 144.3 AU/cm^2, was incorporated into the gelatin solution and coated onto the TPS/PBAT film. Both TPS/PBAT films containing LAE and 69.4 and 144.3 AU/cm^2

Table 3
Antibacterial activity of various lauric arginate (LAE) coating methods on thermoplastic starch/polybutylene adipate terephthalate (TPS/PBAT) films.

Bacterial indicators	Area of the inhibition zone (mm^2) of LAE (0.16 mg/cm^2)-coated TPS/PBAT[1]		
	Direct coating	Incorporating with pullulan	Incorporating with gelatin
S. Typhimurium ATCC 14028	633.00 ± 12.01$^{(a)}$	835.33 ± 12.34$^{(b)}$	1468.33 ± 2.75$^{(c)}$
S. Enteritidis ATCC 10118	599.67 ± 7.77$^{(a)}$	856.66 ± 11.01$^{(b)}$	1620.00 ± 5.63$^{(c)}$
L. monocytogenes Scott A	825.00 ± 10.54$^{(a)}$	958.67 ± 5.51$^{(a)}$	1335.33 ± 10.41$^{(b)}$
S. aureus ATCC 12600	238.33 ± 15.31$^{(a)}$	330.33 ± 8.14$^{(b)}$	1550.00 ± 5.00$^{(c)}$
E. coli O157:H7 ATCC 43895	669.00 ± 12.76$^{(a)}$	1768.67 ± 27.30$^{(b)}$	2070.00 ± 3.04$^{(c)}$
V. parahaemolyticus ATCC 17802	746.67 ± 7.64$^{(a)}$	1901.33 ± 48.01$^{(b)}$	2168.30 ± 2.75$^{(c)}$

[1] Values in the same row followed by different letters significantly differed ($P < 0.05$).

Table 4
Antibacterial activity of gelatin containing lauric arginate (LAE; 0.16 mg/cm²) and nisin Z (0.0–144.3 AU/cm²)-coated on thermoplastic starch/polybutylene adipate terephthalate (TPS/PBAT) film.

Bacterial indicators	Area of the inhibition zone (mm²) of gelatin containing LAE (0.16 mg/cm²) and nisin Z-coated on TPS/PBAT film[1]			
	0.0 AU/cm²	34.6 AU/cm²	69.4 AU/cm²	144.3 AU/cm²
S. Typhimurium ATCC 14028	1153.33 ± 55.08[(ab)]	1120 ± 26.46[(a)]	1240 ± 51.96[(bc)]	1280 ± 34.64[(c)]
S. Enteritidis ATCC 10118	1383.33 ± 28.87[(a)]	1413.33 ± 55.08[(a)]	1460.00 ± 34.64[(a)]	1480.00 ± 60.83[(a)]
L. monocytogenes Scott A	1113.33 ± 57.73[(a)]	1166.67 ± 76.38[(a)]	1476.67 ± 32.14[(b)]	1603.33 ± 55.08[(b)]
S. aureus ATCC 12600	1466.67 ± 57.73[(a)]	1460.00 ± 34.64[(a)]	1606.67 ± 55.08[(b)]	1633.33 ± 28.86[(b)]
E. coli O157:H7 ATCC 43895	1793.33 ± 40.41[(a)]	1816.67 ± 76.38 [(a)]	1950.00 ± 50.00[(b)]	2086.67 ± 32.15[(c)]
V. parahaemolyticus ATCC 17802	2050.00 ± 50.00[(a)]	2116.67 ± 28.87[(a)]	2216.67 ± 28.87[(b)]	2286.67 ± 63.51[(b)]

[1] Values in the same row followed by different letters significantly differed ($P < 0.05$).

displayed excellent inhibition of all test strains, including *E. coli* O157:H7 ATCC 43895, *L. monocytogenes* Scott A, *S.* Typhimurium ATCC 14028, *S.* Enteritidis ATCC 10118 and *V. parahaemolyticus* ATCC 17802. However, the antimicrobial activities of TPS/PBAT films containing LAE and 69.4 and 144.3 AU/cm² of nisin Z against all test strains were not significantly different ($P > 0.05$), except for *E. coli* O157:H7 ATCC 43895 (Table 4). Pattanayaiying et al. (2015) also demonstrated that pullulan film with the combination of LAE and nisin Z was active against *Brochothrix thermosphacta* DSM 20171, *E. coli* O157:H7 ATCC 43895, *E. coli* O111, *E. coli* O26, *Listeria monocytogenes* Scott A, *S.* Typhimurium ATCC 14028, *S.* Typhimurium ATCC 13311, *S.* Enteritidis ATCC 10118 and *S. aureus* in a plate overlay assay. In this study, the active TPS/PBAT film containing nisin Z also was compared to the antimicrobial activity with the film containing commercial nisin (Sigma) at the same concentration. The antimicrobial activity of the films produced by coating with different sources of nisin was not significantly different against all test strains ($P > 0.05$), except for *V. parahaemolyticus*, which the commercial nisin coating on TPS/PBAT film was inhibited more than nisin Z (Table 5). As a result, the active TPS/PBAT film was prepared by coating of the gelatin polymer containing LAE (0.08 mg/cm²) alone (LAE-Gelatin-TPS/PBAT film) or in a combination with nisin Z (69.4 AU/cm²) on TPS/PBAT film (Nisin-LAE-Gelatin-TPS/PBAT film) for the subsequent challenge study.

3.2. Effect of the active TPS/PBAT films against foodborne pathogens associated with seafood during refrigerated storage

3.2.1. Inhibition of Salmonella

Remaining populations of *S.* Typhimurium ATCC 14028 on refrigerated seafood, including bigeye snapper and tiger prawn slices treated with TPS/PBAT, LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films are shown in Fig. 1A and B, respectively. The inoculated seafood without any film treatment (non-film) also was sealed, refrigerated, sampled, and determined for the remaining populations of *S.* Typhimurium ATCC 14028.

3.2.1.1. Inhibition of Salmonella Typhimurium on refrigerated, raw bigeye snapper slices. The inoculated raw bigeye snapper slices without film treatment (non-film) and with TPS/PBAT film (control) allowed *S.* Typhimurium to grow on surfaces of raw bigeye snapper slices, particularly during the first 3 days of the refrigerated storage. Then, the populations gradually decreased and remained stable at 7.3–7.4 log CFU/g after 14 days of storage (Fig. 1A). *Salmonella* spp. populations associated with raw bigeye snapper slices treated with LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films were not significantly different. Both active TPS/PBAT films eliminated *Salmonella* spp. populations 3.2–3.51 $\log_{10}$ CFU/g from raw bigeye snapper slices, as compared with the control film and non-film treatment at 21 days of refrigerated storage (Fig. 1A). Theinsathid et al. (2012) also demonstrated that *S.* Typhimurium on sliced ham was reduced over 3 log orders from 5.7 to 2 to 2.5 $\log_{10}$ CFU/film coating with 2.6% (w/w) LAE. Guo et al. (2014) also indicated that polylactic acid (PLA) film containing 1.94 mg/cm² of LAE reduced 1–1.5 $\log_{10}$ of *S.* Typhimurium populations on ready-to-eat meat during 3 and 5 weeks storage at 10 °C, as compared with the control. Moreover, Pattanayaiying et al. (2015) also reported that pullulan film containing LAE and the combination of LAE with nisin Z reduced 2.5–4.5 $\log_{10}$ CFU/cm² and 3.5–5.1 $\log_{10}$ CFU/cm² *Salmonella* spp. populations, respectively.

3.2.1.2. Inhibition of Salmonella on refrigerated raw tiger prawn slices. *Salmonella* spp. populations on raw tiger prawn slices treated with Nisin-LAE-Gelatin-TPS/PBAT films were dramatically decreased and eliminated from raw tiger prawn slices after 14 days of refrigerated storage (Fig. 1B). However, the LAE-Gelatin-TPS/PBAT film also eliminated *Salmonella* spp. populations from raw tiger prawn slices after storage at 4 °C for 21 days (Fig. 1B).

Salmonella spp. populations in raw tiger prawn slices treated with the gelatin-coated TPS/PBAT film without an antimicrobial agent (Gelatin-TPS/PBAT film; control film) were inhibited 1.4 $\log_{10}$ CFU/g following three days of refrigerated storage. Subsequently, the

Table 5
Antimicrobial activity of gelatin containing lauric arginate (LAE; 0.08 mg/cm²) alone or combination with nisin Z (69.4 AU/cm²)-coated on thermoplastic starch/polybutylene adipate terephthalate (TPS/PBAT) film.

Bacterial indicators	Area of the inhibition zone (mm²) of active Gelatin-TPS\PBAT bilayer film[1]			
	Gelatin	Gelatin + LAE	Gelatin + LAE + nisin Z	Gelatin + LAE + nisin (Sigma)
S. Typhimurium ATCC 14028	113.67 ± 6.35[(a)]	1153.33 ± 55.08[(b)]	1240.00 ± 51.96[(b)]	1220.00 ± 26.46[(b)]
S. Enteritidis ATCC 10118	110 ± 0.00[(a)]	1383.33 ± 28.87[(b)]	1460.00 ± 34.64[(c)]	1466.67 ± 30.55[(c)]
L. monocytogenes Scott A	115.67 ± 5.51[(a)]	1113.33 ± 57.73[(b)]	1476.67 ± 32.14[(c)]	1400.00 ± 0.00[(c)]
S. aureus ATCC 12600	115.00 ± 5.56[(a)]	1466.67 ± 57.73[(b)]	1606.67 ± 55.08[(b)]	1550.00 ± 50.00[(b)]
E. coli O157:H7 ATCC 43895	108.67 ± 2.31[(a)]	1793.33 ± 40.41[(b)]	1950.00 ± 50.00[(c)]	1883.3 ± 28.86[(c)]
V. parahaemolyticus ATCC 17802	116.33 ± 5.67[(a)]	2050.00 ± 50.00[(b)]	2216.67 ± 28.87[(c)]	2016.67 ± 76.37[(b)]

[1] Values in the same row followed by different letters significantly differed ($P < 0.05$).

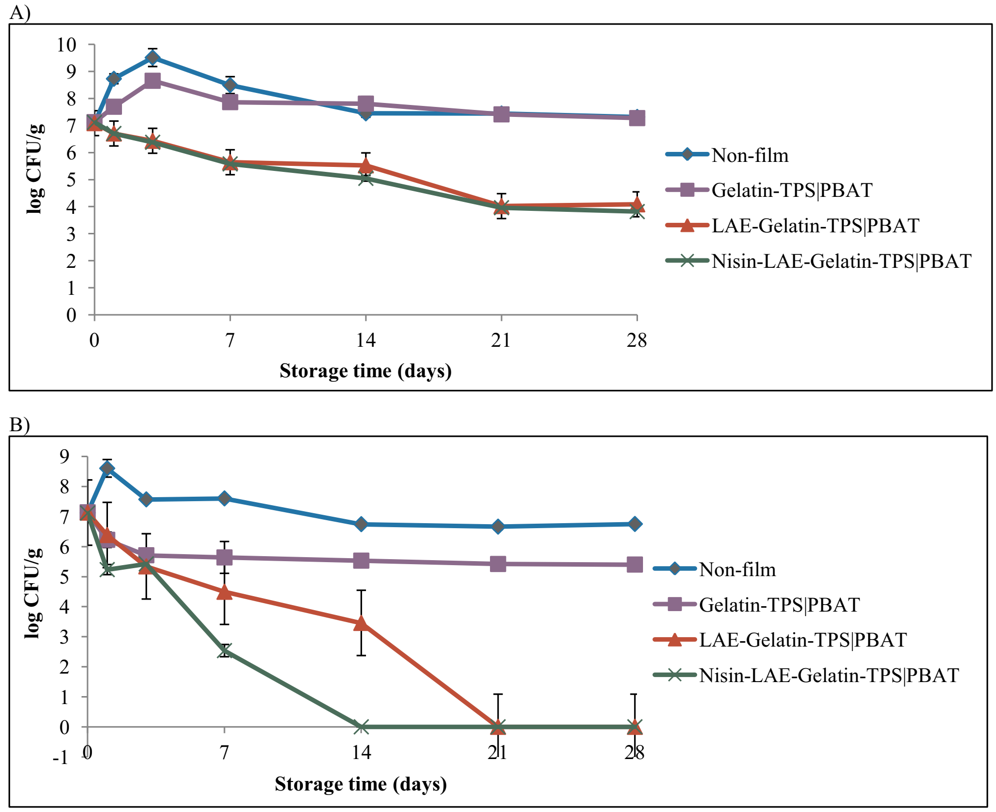

Fig. 1. Remaining populations of *Salmonella* Typhimurium ATCC 14028 on inoculated bigeye snapper (A) and tiger prawn (B) slices either treated with different films or non-film treatment during storage at 4 °C up to 28 days.

population of *S.* Typhimurium was stable through 28 days of storage (Fig. 1B). The slight reduction of *Salmonella* spp. populations might result from gelatin on TPS/PBAT film which demonstrated low antimicrobial activity in the preliminary treatment (data not shown) and vacuum packaging. Conversely, *Salmonella* populations in raw tiger prawn slices without film treatment (non-film) increased from 7.1 to 8.6 $\log_{10}$ CFU/g following the first day of refrigerated storage. Then, the population decreased slightly and remained at 6.7 $\log_{10}$ CFU/g by the end of the experiment.

Conversely, the remaining populations of *S.* Typhimurium on raw bigeye snapper slices with all treatments were higher and less sensitive to the storage and antimicrobials than the populations associated with raw tiger prawn slices. These results suggest that bigeye snapper slices may be comprised of higher nutrients for microbial growth than tiger shrimp slices (CalorieSlism, 2017).

3.2.2. Inhibition of *V. parahaemolyticus*

Remaining populations of *V. parahaemolyticus* ATCC 17802 on refrigerated raw bigeye snapper and tiger prawn slices treated with Gelatin-TPS/PBAT film, LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films are shown in Fig. 2A and B, respectively. The inoculated seafood without film treatment (non-film) also was sealed, refrigerated, sampled and remaining populations of *V. parahaemolyticus* ATCC 17802 determined.

3.2.2.1. Inhibition of V. parahaemolyticus on refrigerated raw bigeye snapper slices. The populations of *V. parahaemolyticus* on raw bigeye snapper slices without film treatment (non-film) were stable throughout the experiment (Fig. 2A). Meanwhile, the control film inhibited the population of *V. parahaemolyticus* on raw bigeye snapper slices 1.1 $\log_{10}$ CFU/g after the first day of treatment. Then, the population was slightly increased (0.9 $\log_{10}$ CFU/g) by the end of the refrigerated storage (Fig. 2A). The populations of *V. parahaemolyticus* on raw bigeye snapper slices treated with the antimicrobial coating TPS/PBAT film decreased. The Nisin-LAE-Gelatin-TPS/PBAT film reduced *V. parahaemolyticus* populations from 6.2 to 1.8 $\log_{10}$ CFU/g throughout the experiment (Fig. 2A). The LAE-Gelatin-TPS/PBAT film reduced *V. parahaemolyticus* population only 2.7 $\log_{10}$ CFU/g during 28 days of storage (Fig. 2A).

3.2.2.2. Inhibition of V. parahaemolyticus on refrigerated raw tiger prawn slices. The populations of *V. parahaemolyticus* on refrigerated raw tiger prawn slices were decreased when wrapped with the Gelatin-TPS/PBAT film (control film) and non-film treatment as well (Fig. 2B). The results suggest that tiger prawn slices might be an unsuitable nutrient source for microbial growth and the storage conditions might not be suitable for *V. parahaemolyticus*. Vasudevan et al. (2002) also reported that *V. parahaemolyticus* populations on fish fillets was decreased during storage at 4 °C. Additionally, the results indicated that *V. parahaemolyticus* is more sensitive to gelatin and the storage condition than *Salmonella* Typhimurium (Figs. 1B and 2B). Gelatin-TPS/PBAT film and non-film treatment reduced *V. parahaemolyticus* 3.5 $\log_{10}$ CFU/g on raw tiger prawn slices during 28 days of refrigerated storage (Fig. 2B). Meanwhile, Gelatin-TPS/PBAT film treatment reduced *Salmonella* Typhimurium only 7.3 $\log_{10}$ CFU/g and non-film treatment hardly reduced *Salmonella* Typhimurium on raw tiger prawn slices during 28 days of refrigerated storage (Fig. 1B).

The population of *V. parahaemolyticus* was reduced over 7 $\log_{10}$ CFU/g and eliminated from the inoculated raw tiger prawn slices when wrapped with the gelatin containing antimicrobial(s)-coated TPS/PBAT films; either LAE-Gelatin-TPS/PBAT or Nisin-LAE-Gelatin-TPS/PBAT, followed by vacuum packaging, and long term refrigerated storage (Fig. 2B). Chaiyakosa et al. (2007) reported reductions of *V. parahaemolyticus* on inoculated shrimp were 81 and 96% after applying 0.25% chitosan solutions for 10 and 30 min, respectively. Chappparo-Hernandez et al. (2015) also demonstrated that chitosan, in

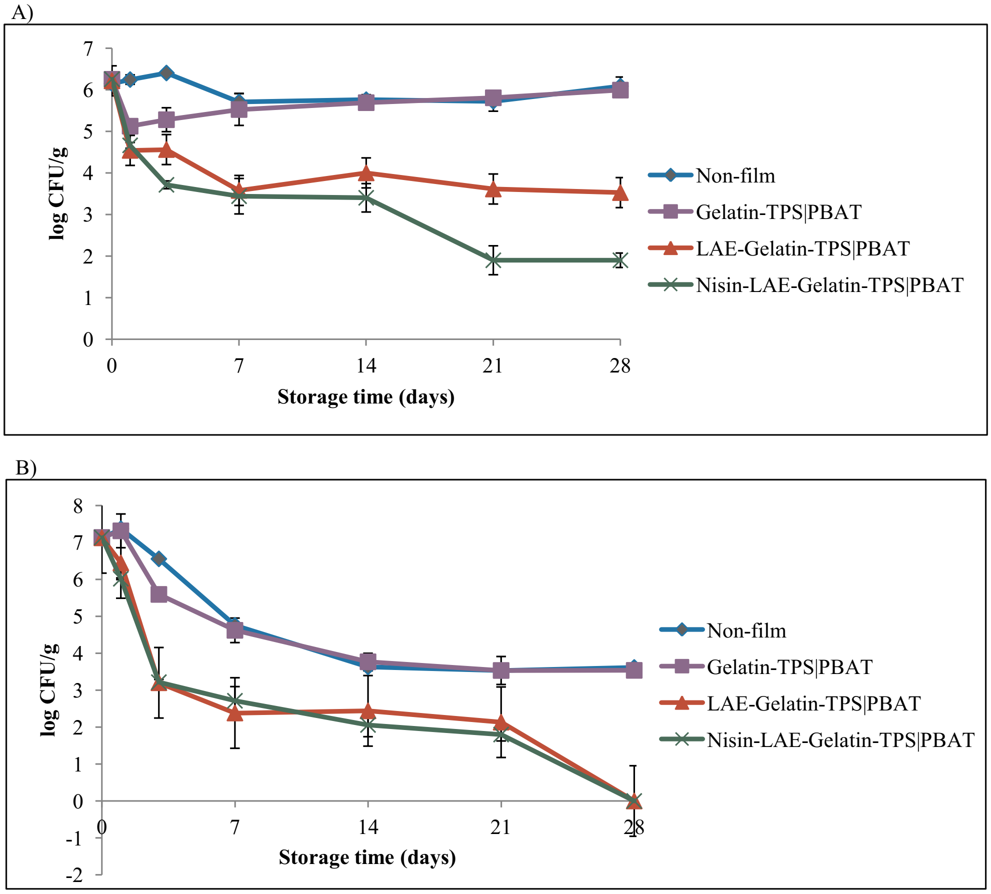

Fig. 2. Remaining populations of *Vibrio parahaemolyticus* ATCC 17803 on inoculated bigeye snapper (A) and tiger prawn (B) slices either treated with different films or non-film treatment during storage at 4 °C up to 28 days.

combination with 0.25% carvacrol, reduced *V. parahaemolyticus* populations 3.1 $\log_{10}$ CFU/g on tilapia fillets during 20 days of ice storage, when compared with the control. Meanwhile, Terzi and Gucukoglu (2010) obtained a 33% reduction of *V. parahaemolyticus* in the inoculated mussels when treated with 0.05% chitosan for 30 min.

3.3. Effect of the active TPS/PBAT films against foodborne pathogens associated with seafood during frozen storage

3.3.1. Inhibition of Salmonella

Remaining populations of *S.* Typhimurium ATCC 14028 on frozen seafood, including bigeye snapper and tiger prawn slices wrapped with Gelatin-TPS/PBAT film, LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT are shown in Fig. 3A and B, respectively. The inoculated seafood without film (non-film) also was sealed, refrigerated, sampled and determined for the remaining *S.* Typhimurium ATCC 14028.

3.3.1.1. Inhibition of Salmonella on frozen raw bigeye snapper slices. All treatments of bigeye snapper slices with the films reduced *Salmonella* spp. populations after storage at −20 °C. The results suggest that frozen temperatures might destroy bacterial cells. The *Salmonella* spp. populations on raw bigeye snapper slices without film (non-film) and with Gelatin-TPS/PBAT film (control) were reduced 2.2 and 2.8 $\log_{10}$ CFU/g, respectively, throughout the experiment (Fig. 3A). *Salmonella* spp. populations on raw bigeye snapper slices wrapped with the LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films were eliminated 5.7 $\log_{10}$ CFU/g by day 60 of frozen storage (Fig. 3A). Furthermore, the LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films reduced 3.6 or 2.96 $\log_{10}$ CFU/g of *Salmonella* spp. population as compared with the non-film treatment and Gelatin-TPS/PBAT film (control) at 60 days of frozen storage, respectively (Fig. 3A).

3.3.1.2. Inhibition of Salmonella on frozen raw tiger prawn slices. The *Salmonella* spp. populations on raw tiger prawn shrimp slices without film (non-film) and with Gelatin-TPS/PBAT film (control) were decreased approximately 2.8–2.9 $\log_{10}$ CFU/g after storage at −20 °C for 60 days (Fig. 3B). The LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films eliminated *Salmonella* populations on raw tiger prawn slices after storage at −20 °C for 21 and 7 days, respectively (Fig. 3B). *S.* Typhimurium on tiger prawn samples was more sensitive to the films with coated antimicrobial(s) than the *Salmonella* associated with the bigeye snapper. These results suggest that tiger prawns may lack nutrients needed for bacterial growth, while the bigeye snapper is nutrient rich (CalorieSlism, 2017).

3.3.2. Inhibition of V. parahaemolyticus

Remaining population of *V. parahaemolyticus* ATCC 17802 on frozen raw bigeye snapper and tiger prawn slices wrapped with Gelatin-TPS/PBAT film, LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films are shown in Fig. 4A and B, respectively. The inoculated seafood without film wrapping (non-film) also was sealed, refrigerated, sampled and determined for the remaining populations of *V. parahaemolyticus* ATCC 17802.

3.3.2.1. Inhibition of V. parahaemolyticus on frozen raw bigeye snapper slices. V. parahaemolyticus populations were eliminated from raw bigeye snapper slices without film and with Gelatin-TPS/PBAT film (control) wrapping after storage at −20 °C for 60 days (Fig. 4A). These results suggest that the frozen temperature and vacuum-packaged

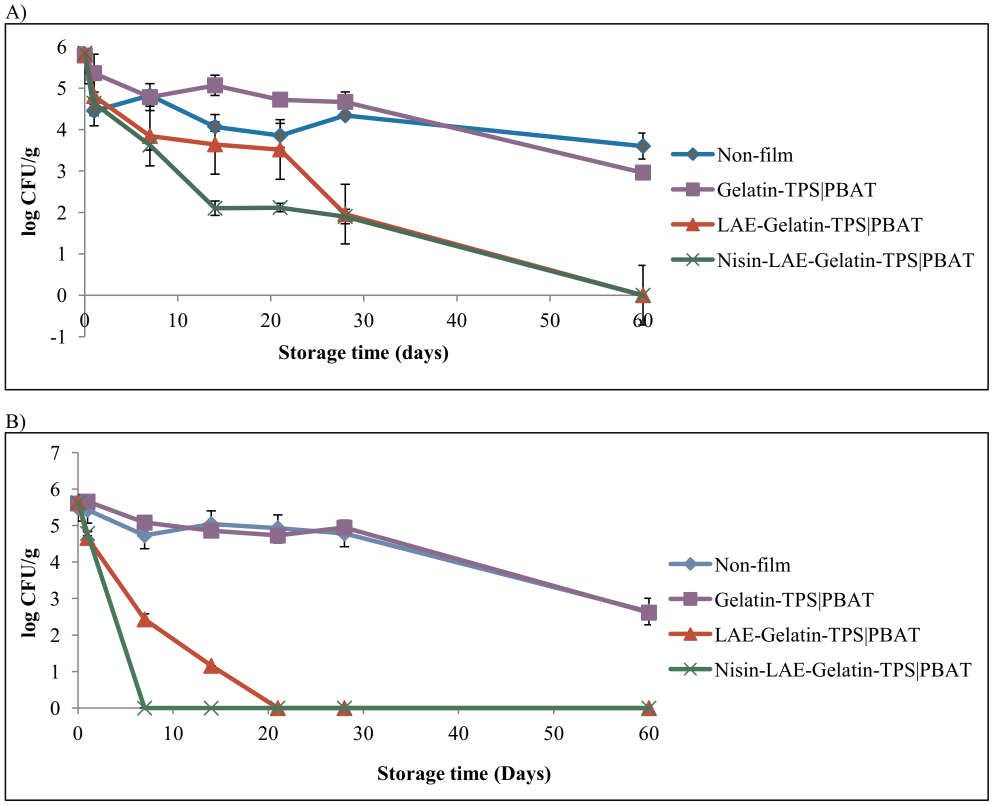

Fig. 3. Remaining populations of *Salmonella* Typhimurium ATCC 14028 on inoculated bigeye snapper (A) and tiger prawn (B) slices either treated with different films or non-film treatment during storage at −20 °C up to 60 days.

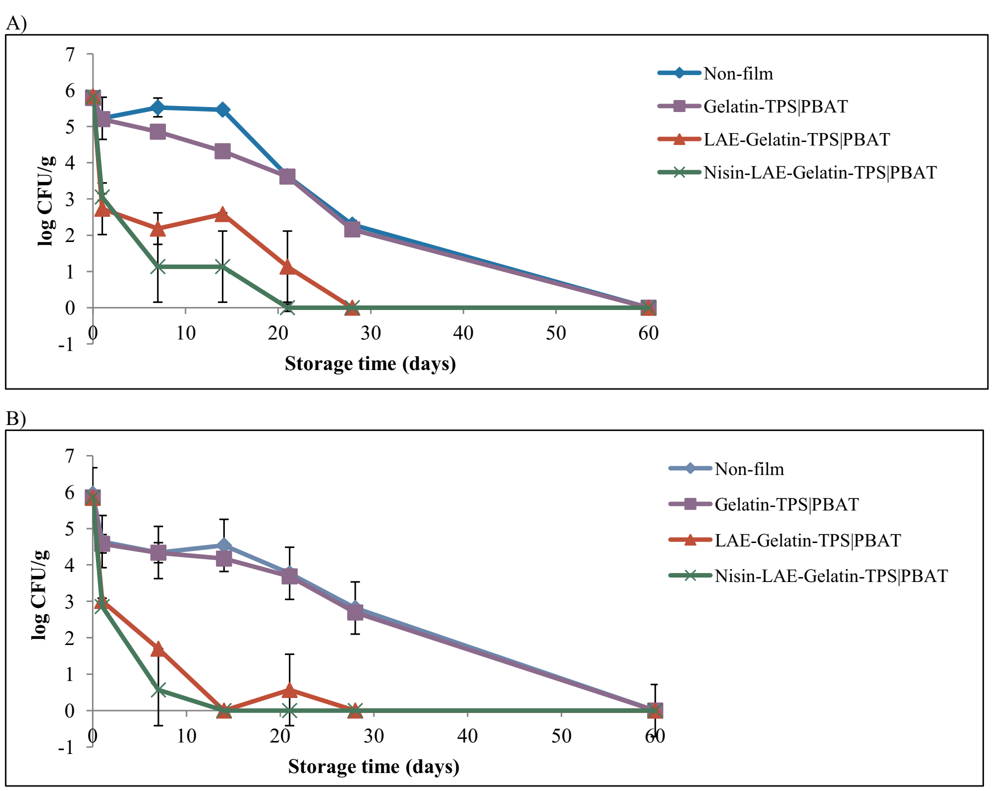

Fig. 4. Remaining populations of *V. parahaemolyticus* ATCC 17802 on inoculated bigeye snapper (A) and tiger prawn (B) slices either treated with different films or non-film treatment during storage at −20 °C up to 60 days.

conditions may destroy bacterial cells. The LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films appeared to eliminate *V. parahaemolyticus* from the bigeye snapper slice after storage at −20 °C for 28 and 21 days (Fig. 4A), respectively. Since *V. parahaemolyticus* is more susceptible to freezing than *S.* Typhimurium, it was reduced faster than *S.* Typhimurium on frozen raw bigeye snapper slices.

3.3.2.2. Inhibition of V. parahaemolyticus on frozen raw tiger prawn slices. As with raw bigeye snapper, *V. parahaemolyticus* populations were eliminated from raw tiger prawn slices without film (non-film) and with TPS/PBAT film (control) wrapping after storage at −20 °C for 60 days (Fig. 4B). LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films dramatically decreased *V. parahaemolyticus* population on tiger prawn slices (Fig. 4B). The results are not surprising because *V. parahaemolyticus* is a fragile pathogen and it was inoculated onto tiger prawn shrimp with limited nutrients, under frozen and vacuum-packaged storage. These conditions appear to be synergistic and can enhance the antimicrobial activity of the active TPS/PBAT films. Therefore, 5.8 $\log_{10}$ CFU/g of the initial *V. parahaemolyticus* populations were eliminated from raw tiger prawn slices when wrapped with LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films after storage at −20 °C for 28 and 14 days, respectively (Fig. 4B).

4. Conclusions

Our findings demonstrate that the antimicrobial TPS/PBAT films, LAE-Gelatin-TPS/PBAT and Nisin-LAE-Gelatin-TPS/PBAT films effectively inhibited *V. parahaemolyticus* ATCC17802 and *S.* Typhimurium ATCC14028 on bigeye snapper (*L. lineolatus*) and tiger prawn (*P. monodon*) slices during long-term refrigerated and frozen (−20 °C) storage. Nisin-LAE-Gelatin-TPS/PBAT film was more effective against both foodborne pathogens than LAE-Gelatin-TPS/PBAT film; consequently, it might be advantageous for the control of foodborne pathogens associated with seafood products.

Acknowledgments

This research was financially supported by the Biodiversity-Based Economy Development Office (Public Organization), Bangkok, Thailand.

References

Allshouse, J., Buzby, J., Harvey, D., Zorn, D., 2004. Seafood Safety and Trade. Agriculture Information Bulletin Number 789-7. United States Department of Agriculture Economic Research Service.

Amagliani, G., Brandi, G., Schiavano, G.F., 2012. Incidence and role of *Salmonella* in seafood safety. Food Res. Int. 45, 780–788.

Bakal, G., Diaz, A., 2005. The lowdown on lauric arginate: food antimicrobial hammers away at plasma membrane, disrupting a pathogen's metabolic process. Food Qual. 12, 54–61.

Bakr, W.M., Hazzah, W.A., Abaza, A.F., 2011. Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. J. Am. Sci. 7, 663–668.

Brandelero, R.P.H., Grossmann, M.V., Yamashita, F., 2012. Films of starch and poly(butylene adipate co-terephthalate) added of soybean oil (SO) and tween 80. Carbohydr. Polym. 90, 1452–1460.

CalorieSlism, 2017. Food nutrition facts and count calories in food. Retrieved from. http://slism.com/calorie/.

Chaiyakosa, S., Charernjiratragul, W., Umsakul, K., Vuddhakul, V., 2007. Comparing the efficiency of chitosan with chlorine for reducing *Vibrio parahaemolyticus* in shrimp. Food Control 18, 1031–1035.

Chappparo-Hernandez, S., Ruiz-Cruz, S., Marquez-Rios, E., Ocana-Higuera, V.M., Valenzuela-Lopez, C.C., Ornelas-Paz, J.J., Del-Toto-Sanchez, C.L., 2015. Effect of chitosan-carvacrol edible coatings on the quality and shelf life of tilapia (*Oreochromis niloticus*) fillets stored in ice. Food Sci. Technol. 35, 734–741.

Cuadrado, B.S., Viedma, P.M., Aguayo, M.C.L., Blazquez, I.O., Burgos, M.J.G., Pulido, R.P., Galvez, A., Lopez, R.L., 2016. Effect of activated plastic films on inactivation of foodborne pathogens. Coatings 6, 8.

Cutter, C.N., Siragusa, G.R., 1998. Incorporation of nisin into a meat binding system to inhibit bacteria on beef surfaces. Lett. Appl. Microbiol. 27, 19–23.

Emiroglu, A.K., Yemis, G.P., Coskun, B.K., Candogan, K., 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci. 86, 283–288.

FDA, 2001. GRAS Notice No. GRN 000065. Agency Response Letter From FDA. (April, 2001).

FDA, 2005. GRAS Notice No. GRN 000164. Non-objection Letter From FDA. (September, 2005).

Fonseka, T.S.G., 1990. Microbial flora of pond cultured prawn (*Penaeus monodon*). FAO Fish. Rep. 401, 24–31.

Guo, M., Jin, T.Z., Yang, R., 2014. Antimicrobial poly lactic acid packaging films against *Listeria* and *Salmonella* in culture medium and on ready-to-eat meat. Food Bioprocess Technol. 7, 3293–3307.

Hibbeln, J.R., Davis, J.M., Steer, C., Emmett, P., Rogeers, I., William, C., Golding, J., 2007. Maternal seafood consumption in pregnancy and neuro-development outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 269, 578–585.

Huang, Y., Ghate, V., Phua, L., Yuk, H.-G., 2012. Prevalence of *Salmonella* and *Vibrio* spp. in seafood product sold in Singapore. J. Food Prot. 75, 1320–1323.

Huss, H.H., 1997. Control of indigenous pathogenic bacteria in seafood. Food Control 8, 91–98.

Iwamoto, M., Ayer, T., Mahon, B.E., Swerdlow, D.L., 2010. Epidemiology of seafood-associated infections in the United States. Clin. Microbiol. Rev. 23, 399–411.

Kashiri, M., Cerisuelo, J.P., Dominguez, I., Lopez-Carballo, G., Hernandez-Munoz, P., Gavara, R., 2016. Novel antimicrobial zein film for controlled release of lauroyl arginate (LAE). Food Hydrocoll. 61, 547–554.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J., 2002. Fish consumption, fish oil omega-3 fatty acids and cardiovascular disease: AHA scientific statement. Circulation 106, 2747–2757.

Kumar, H.S., Sunil, R., Venugopal, M.N., Karunasagar, I., Karunasagar, I., 2003. Detection of *Salmonella* spp. in tropical seafood by polymerase chain reaction. Int. J. Food Microbiol. 88, 91–95.

Letchumanan, V., Chan, K.-G., Lee, L.-H., 2014. *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advance molecular identification techniques. Front. Microbiol. 5, 1–13.

Min, B.J., Han, I.Y., Dawson, P.L., 2010. Antimicrobial gelatin films reduce *Listeria monocytogenes* on turkey bologna. Poult. Sci. 89, 1307–1314.

Nelapati, S., Nelapati, K., Chinnam, B.K., 2012. *Vibrio parahaemolyticus* – an emerging foodborne pathogen—a review. Vet. World 5, 48–62.

Olgunoglu, I.A., 2012. In: Mahmoud, B.S.M. (Ed.), Salmonella – A Dangerous Foodborne Pathogen. InTech, Croatia.

Pattanayaiying, R., 2015. Isolation and Identification of Lactic Acid Bacteria From Fish Intestine, Characterization of the Bacteriocin, Nisin Z I8-7-3, and Incorporation of the Bacteriocin into Pullulan Films to Control Foodborne Microorganisms. Prince of Songkla University, Songkhla, Thailand (Unpublished doctoral dissertation).

Pattanayaiying, R., Hongpattarakere, T., H-Kittikun, A., 2012. Bacteriocins of lactic acid bacteria from gut of walking catfish with activity against foodborne pathogens and food spoilage bacteria. In: Poster Session Presented at: System Biotechnology: Quality & Success. The 23rd Annual Meeting of the Thai Society of Biotechnology, (2012 Feb 1–2; Bangkok, Thailand).

Pattanayaiying, R., H-Kittikun, A., Cutter, C.N., 2014. Effect of lauric arginate, bacteriocin I8-7-3 and a combination against several food-related bacteria. Int. J. Food Microbiol. 188, 135–146.

Pattanayaiying, R., H-Kittikun, A., Cutter, C.N., 2015. Incorporation of nisin Z and lauric arginate into pullulan films to inhibit foodborne pathogens associated with fresh and ready-to-eat muscle foods. Int. J. Food Microbiol. 207, 77–82.

Ramos, M., Valdes, A., Beltran, A., Garrigos, M.C., 2016. Gelatin-based films and coatings for food packaging applications. Coatings. 6, 41.

Rattagol, P., Wongchinda, N., Sanghtong, N., 1990. *Salmonella* contamination in Thai shrimp. FAO Fish. Rep. 401, 18–23.

Shabarinath, S., Kumar, H.S., Khushiramani, R., Karunasagar, I., Karunasagar, I., 2007. Detection and characterization of *Salmonella* associated with tropical seafood. Int. J. Food Microbiol. 114, 227–233.

Terzi, G., Gucukoglu, A., 2010. Effects of lactic acid and chitosan on the survival of *V. parahaemolyticus* in mussel samples. J. Anim. Vet. Adv. 9, 990–994.

The Target Group, Inc, 2012. Lauric arginate: a new active preservative derived from natural materials. http://www.foodsafetymagazine.com/articlePF.asp?id=4286&sub=sub1.

Theinsathid, P., Visessanguan, W., Kruenate, J., Kingcha, Y., Keeratipibul, 2012. Antimicrobial activity of lauric arginate-coated polylactic acid films against *Listeria monocytogenes* and *Salmonella* Typhimurium on cooked sliced ham. J. Food Sci. 77, 142–149.

Trinetta, V., Floros, J.D., Cutter, C.N., 2010. Sakacin A-containing pullulan film: an active packaging to control epidemic clones of *Listeria monocytogenes* in ready-to-eat foods. J. Food Saf. 30, 366–381.

Vasudevan, P., Marek, P., Daigle, S., Hoagland, T., Venkitanarayanan, K.S., 2002. Effect of chilling and freezing on survival of *Vibrio parahaemolyticus* on fish fillets. J. Food Saf. 22, 209–217.

WHO, 2001. Report of the Joint FAO/WHO expert consultation on risk assessment of microbiological hazards in foods. In: Hazard Identification, Exposure Assessment and Hazard Characterization of *Campylobacter* spp. in Broiler Chickens And *Vibrio* spp. in Seafood. WHO Headquarters, Geneva, Switzerland (23–27 July 2001).

Yang, X., Wu, Qingping, Zhang, J., Huang, J., Chen, L., Liu, S., Yu, S., Cai, S., 2015. Food Control 57, 308–313.