Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

Original Research Article

Antimicrobial activity of oil extracted from *Rana tigerina* skin and its impact on gelatin-based film

Supatra Karnjanapratum[1]*, Passakorn Kingwascharapong[2] and Sitthipong Nalinanon[1]

[1]Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Road, Ladkrabang, Bangkok 10520 Thailand
[2]Laboratory of Postharvest Science, Faculty of Agriculture, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

ARTICLE INFO

Article history:
Received 31 July 2018
Received in revised form 31 December 2018
Accepted 08 January 2019

Keywords:
Antimicrobial activity
Frog skin
Gelatin-based film
Oil
Rana tigerina

ABSTRACT

Oil from the Asian bullfrog (*Rana tigerina*) skin (FSO) was extracted and evaluated for its antibacterial activities against food-borne pathogenic bacteria. The FSO showed inhibitory activities on both Gram +ve (*Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*) and Gram -ve (*Escherichia coli*, *Salmonella* Typhimurium and *Vibrio cholera*) bacteria with minimum inhibitory concentrations (MIC) ranged from 0.059-0.469 mg/mL; on the other hand, those bacteria could not be inhibited by palm oil. Gelatin film solutions emulsified with FSO (FSOS) or palm oil (POS) were tested for antimicrobial activity. The result showed that emulsion system of FSOS had impact on inhibitory activity. FSOS exhibited the higher activity with the lower MIC ($p<0.05$), compared to that of FSO. However, MIC obtained from FSOS increased for *S. aureus* and *S.* Typhimurium. This might indicate the loss of antimicrobial availability of FSO when incorporated with gelatin film solution. Characteristics of the FSO emulsion gelatin-based film (FSOF) were evaluated in comparison with those of palm oil emulsion film (POF). FSOF showed the higher intense of yellow color, compared with those of POF ($p<0.05$). Two different assays, suspension culture medium (SCM) and disk-diffusion (DD) assays, were conducted to determine the antimicrobial activity of FSOF and POF. Based on SCM method, FSOF showed the potential on microbial growth inhibition against all bacterial tested by decreasing the bacterial count number. Nevertheless, there was no inhibition zone detected, when DD was applied. These could be accounted that the oil might be trapped in the film matrix and limited the release of the oil into agar. Therefore, the oil extracted from *R. tigerina* skin could be an excellent natural alternative antimicrobial agent against both Gram +ve and Gram -ve bacteria, which could be develop and use as antimicrobial film packaging.

* *Corresponding author*: Tel.: +66-082-3293652
E-mail address: supatra.ka@kmitl.ac.th

Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

INTRODUCTION

There are several species of frogs such as American Bullfrog, *Lithobates catesbeianus* and the Indian Tiger frog, *Hoplobatrachus tigrinus* (Daszak, Schloege, Louise, Cronin, Pokras, Smith, et al., 2006) and others are bred for consumption or medicinal uses (Oduntan, O., & Jenyo-Oni, 2012). Asian bullfrog (*Rana tigerina*) is commonly farmed in many parts of Thailand for domestic consumption and export (Pariyanonth & Daorerk, 1994). Frog farming has expanded throughout Thailand due to the productive culture and market demand. Frog production was reported to be approximately 10 tonnes per day for both local and oversea markets, particularly Hong Kong, Singapore and Taiwan (Wongtavatchai, Rungsipipat, Chumkaeo, & Surachetpong, 2003). Up to 45% of the frog body parts ultimately go to the waste without being properly utilized for useful purposes. This waste, particularly frog skin, has been used for making crispy skin for domestic consumption.

Antimicrobial activity of fatty acids has been reported from several sources including algae, diatoms and fish. Alencar, Xavier-Júnior, Morais, Dantas, Dantas-Santos, Verissimo, et al. (2015) studied antimicrobial activity of nanostructured emulsion based on *R. catesbeiana* oil against fungi and bacteria related to skin diseases. *R. catesbeiana* oils could be a promising candidate for the treatment of infections and may be used to incorporate as antimicrobial drugs. Oil from *R. catesbeiana* frog skins can contain antimicrobial compounds, but there was no information on *R. tigerina*'s antimicrobial activity. This frog skin, by-product from cutting process, would be a great source for bioactive compounds, especially extracted oil with antibacterial activity.

Emulsion systems have been applied in protein-based gelatin films to improve their physical, barrier and bioactivity properties. Several oils including essential oils from different sources have been used for film preparation (Tongnuanchan, Benjakul, & Prodpran, 2014; Zinoviadou, Koutsoumanis, & Biliaderis, 2010). Those oils showed antimicrobial and antioxidative properties and could lower water vapor permeability of resulting film. Its incorporation in emulsion gelatin-based film can be performed to produce active packaging for food industry. Moreover, the application of those essential oils in film as active packaging could avoid the interfering of colour and flavour from extracted oil by directly adding to the food product.

Therefore, this study focused on the possibility of using *R. tigerina* skin oil, which is generally a byproduct in frog farming in Thailand, as a new natural preservative for active packaging application.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were of analytical grade. Chloroform, methanol, sodium sulphate and glycerol were obtained from Merck (Darmstadt, Germany). Sodium chloride, tetracycline and soy lecithin (L-α-phosphatidylcholine, HLB~4.0) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from Oleen Co., Ltd. (Samutsakorn, Thailand). Soyabean Casein Digest Medium (Tryptone soya broth), Mueller-Hinton agar and Mueller-Hinton broth were purchased from Himedia (Mumbai, MU, India).

Frog skin preparation

Skins of Asian bullfrog (*Rana tigerina*) weighing 200-300 g each were obtained from a local market in Ladkrabang, Bangkok, Thailand. The skins were washed with iced tap water (1–3°C) and pooled as a composite sample in polyethylene bags and stored at -20°C until used. Prior to oil extraction, frozen skins were cut into small pieces (about 1 × 1 cm^2) using scissors and pulverized in the presence of liquid nitrogen in a blender (Phillips, Guangzhou, China).

Extraction of frog skin oil

Frog skin oil (FSO) was extracted according to the method of (Bligh & Dyer, 1959). Samples (100 g) were homogenised with 800 mL of a mixture of chloroform: methanol: water (1:2:1, v/v/v) at 11,000 rpm for 1 min. To the homogenate, 200 mL of chloroform were added and the mixtures were homogenised for 1 min. Thereafter, 100 mL of water were added and the mixtures were homogenised for 30 s at the same speed. The mixture was filtered through a Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, UK) and the filtrate was transferred into a separating funnel where the chloroform phase (bottom phase) was drained off into an Erlenmeyer flask. Sodium sulphate (anhydrous) (10-12 g) was added and the mixture was shaken thoroughly to remove the residual water. Lipid in chloroform was decanted into a round-bottomed flask through a filter paper (Whatman No. 4). The chloroform was evaporated at 45℃ using a rotary evaporator (Rotavapor, model R-14, Büchi, Tokyo, Japan). The residual solvent was removed by flushing with nitrogen. The lipid was transferred to an amber vial and the sample was kept at -20℃ until analyzed.

Antimicrobial activity of frog skin oil (FSO)

Bacterial culture

Pathogenic bacteria used as test organisms in this study were *Bacillus cereus* DMST 5040, *Escherichia col*i DMST 4212, *Salmonella* Typhimurium DMST 562, *Staphylococcus aureus* DMST 8840 and *Vibrio choleraenon* O1/non O139 DMST 2873 and *Aeromonas hydrophila* TISTR 1321. They were obtained from Department of Medical Sciences, Ministry of Public Health, Thailand (DMST), and Thailand Institute of Scientific and Technological Research (TISTR). The bacterial strains were sub-cultured twice in Trypton Soya broth at 37˚C for 18 h and the density of an inoculum was adjusted to 10^5 CFU/mL before being used (Canillac & Mourey, 2001).

Antibacterial activity of frog skin oil using broth dilution assay

The antibacterial activity of the extracted oil was determined using sterile 96-well plates (Wiegand, Hilpert, & Hancock, 2008). The FSO solution (100 µL, 100 mg/mL in tert-butanol) will serially diluted two-fold using sterilized Tryptone Soya broth (100 µL). A 100 µL of the broth culture (10^5 CFU/mL) was then seeded to each sample well (100 µL) and incubated for 24 h at 37°C. The resulting turbidity was determined by optical density readings at 600 nm and expressed with a using a FLUOstar Omega microplate reader. tert-Butanol at concentrations used in the dilutions was employed as control. The negative control wells were conducted using the serial diluted sample without inoculum. The absorbance values for negative controls were subtracted from the experimental values. The percent growth inhibition was calculated by comparison with a control using the formula indicated below.

$$\text{Growth inhibition (\%)} = (Ac - At)/Ac \times 100$$

where At and Ac are the absorbance of the test group and control group, respectively.

The lowest concentration of the compound that inhibits the growth of the bacterium was determined as the Minimum inhibitory concentrations (MIC) (Kannan, Shanmugavadivu, Petchiammal, & Hopper, 2006).

Impact of frog skin oil on characteristics of emulsion gelatin-based film and its antimicrobial activity

Preparation of film forming solution and emulsion

Film forming emulsion from fish gelatin were prepared according to the method of (Tongnuanchan, Benjakul, Prodpran, Pisuchpen, & Osako, 2016). Gelatin powder was mixed with distilled water to obtain a protein content of 3.5% (wet basis) and the mixture was heated at 70°C for 30 min. Glycerol at a concentration of 30% (w/w, based on protein content) was added into solution. The resulting solution was termed as 'film forming solution'. To prepare the film forming emulsion, frog skin oil (FSO, 50% of oil concentration (w/w, based on protein content)) and Tween-20 (25%, based on oil content), was mixed with film forming solution. The mixture was homogenized at 22,000 rpm for 3 min using a rotor-stator homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The control sample was conducted in the same manner as sample by using palm oil (PO) instead of FSO. The emulsion was referred to 'emulsified film solution' (EFS). EFS were used for film preparation.

Preparation of film

To prepare the films, the EFS (5 g) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown at room temperature for 12 h. The films were further dried at 25°C and 50 ± 5% RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and subjected to analyses. Palm oil/gelatin film and FSO/gelatin film were termed POF and FSOF, respectively.

Film analyses

Film thickness

The thickness of film was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film of ten film samples were used for determination of average thickness.

Color

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, VA, USA). D_{65} (day light) and a measure cell with opening of 30 mm was used. The color of the films was expressed as L^*-value (lightness), a^*-value (redness/greenness) and b^*-value (yellowness/blueness). Total difference of color (ΔE^*) was calculated as follows (Gennadios, Weller, Hanna, & Froning, 1996) :

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

where ΔL^*, Δa^* and Δb^* are the differences between the color parameter of the samples and those of the white standard (L^* = 92.83, a^* = -1.23, b^* = 0.51).

Antibacterial activities

Antibacterial activity of emulsified film solutions (EFS) using broth dilution assay

Antibacterial activity of EFS was determined using broth dilution assay as previously described. EFS was serially diluted two-fold using Tryptone Soya broth to create a concentration sequence from 7.50 to 0.03 mg/ml. The broth culture (100 µL, 10^5 CFU/mL) was then seeded to each sample well (100 µL) and incubated for 24 h at 37°C. The resulting turbidity was determined by optical density readings at 600nm and expressed with a using a FLUOstar Omega microplate reader. The lowest concentration of the compound that inhibits the growth of the bacterium was determined as the MIC. Antimicrobial activity of EFS from PO was tested in the same manner.

Antimicrobial activity of frog skin oil emulsion-based film (FSOF)

Suspension culture medium assay

The suspension culture medium assay was conducted with the selected broth culture (10 mL, 10^5 CFU/ml) with FSOF pieces (sterilized with UV light) in 20 ml sterilized glass vial with screw cap. The FSOF was cut into pieces (2×4 cm^2) before added to the selected broth culture. The vial was then inoculated in shaker incubator (ThermoStable IS-30/-30R, Daihan Scientific Co., Ltd., Gangwon, Korea) at 37°C, 220 rpm for 24 h. The antimicrobial effect of FSOF in the bacterial growth was evaluated from obtained culture broth using plate count agar. The growth number of bacterium was then determined. Antimicrobial activity of POF was tested in the same manner.

Disk-diffusion method

Antimicrobial activity of FSOF was evaluated using disk-diffusion assay (agar spot test) as described by Buntin, Chanthachum, & Hongpattarakere (2018) with slight modification. FSOF was cut into discs (5.5 mm in diameter) using a paper cutter. The tested bacteria strains were inoculated into Muller-Hinton soft medium (MHA). Then the FSOF discs were placed on the inoculated MHA agar. After incubation at 37°C for 24 h, the inhibition zone was examined around the FSOF disc. Antimicrobial activity of POF was tested in the same manner.

Statistical analysis

Experiments were run in triplicate using three lots of samples. Comparison of means was carried out by the independent sample t-test. Statistical analysis was performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Antimicrobial activity of frog skin oil

The effectiveness of both frog skin oil *R. tigerina* skin oil (FSO) and their emulsified film solution (EFS) against food-borne pathogenic bacteria was depicted in Table 1. The resulted showed that both of microorganisms (Gram positive and negative) were inhibited by extracted *R. tigerina* skin oil, regardless of their forms (FSO and EFS). The results indicated the broad spectrum of inhibition activity of extracted *R. tigerina* skin oil. This might relate to lipophilic characteristic of antibacterial agent in FSO, which could aid in their

ability to penetrate cellular and mitochondrial membrane structures. This action leads to membrane disruption, cytoplasmic leakage, cell lysis and death (Munhuweyi, Caleb, Lennox, van Reenen, & Opara, 2017). On the other hand, palm oil, used as a negative control, had no any inhibition effect on all bacteria tested. FSO showed the higher efficiency on inhibiting food-borne pathogenic bacteria, compared with cinnamon essential oil (EO) (Zhang, Liu, Wang, Jiang & Quek, 2016). The minimum inhibition concentration (MIC) of cinnamon EO was similar for both *E. coli* and *Staphylococcus* (1.0 mg/ml). However, the stronger antimicrobial activities against *L. moncytogenes* and *S.* Typhimurium were found for essential oils from oregano and thyme (Mith, Dure, Delcenserie, Zhiri, Daube & Clinquart, 2014). It seem like the FSO is more effectively inhibited more than EFS in all types of tested bacteria than EFS. The dispersed oil as small droplets in emulsion system of EFS might promote the antimicrobial availability of the oil. Nevertheless, the rapid decreases of bacterial growth inhibition activity were found in *S. aureus* and *S.* Typhimurium when testing the oil in form of emulsified film solution (EFS). These could be accounted the different mechanisms and/or active compounds of frog skin oil inhibiting those two strains might be trapped or bound with the film matrix. Consequently, the function of antimicrobial oil was limited. Goldbeck, Victoria, Motta, Savegnago, Jacob, Perin, et al. (2014) found that the essential oil of *Cymbopogon citratus* showed the effectiveness against Gram-positive species better than other pathogenic and spoilage bacteria. Klangmuang and Sothornvit (2016) reported that antimicrobial activity of Thai essential oil was more likely governed by its lipid composition. Therefore, FSO might contain with antimicrobial compound which could inhibit both Gram+ve and Gram -ve food borne pathogenic bacteria.

Table 1. Antimicrobial activity of *R. tigerina* skin oil (FSO) and palm oil (PO) and their emulsified film solutions (EFS) against food-borne pathogenic bacteria using broth dilution assay

Microorganisms	MIC (mg/mL)			
	Oil		EFS	
	FSO	PO	FSO	PO
Gram positive				
Bacillus cereus	0.234[a]	nd.	0.029[b]	nd.
Listeria monocytogenes	0.469[a]	nd.	0.029[b]	nd.
Staphylococcus aureus	0.059[b]	nd.	1.875[a]	nd.
Gram negative				
Escherichia coli	0.469[a]	nd.	0.029[b]	nd.
Salmonella Typhimurium	0.117[b]	nd.	1.875[a]	nd.
Vibrio cholerae non O1/ non O139	0.117[a]	nd.	0.059[b]	nd.

Different superscript letters in the same row indicate significant differences ($p<0.05$). nd; Not detectable. MIC: Minimum inhibitory concentrations

Table 2. Characteristics of gelatin-based film emulsified with *R. tigerina* skin oil (FSOF) and palm oil (POF)

Characteristics	FSOF	POF
Thickness (mm)	0.075±0.002[b, †]	0.086±0.002[a]
Color		
L^*	85.72±0.08[b]	90.08±0.08[a]
a^*	-1.14±0.2[a]	-1.40±0.30[a]
b^*	36.44±0.58[a]	7.95±0.11[b]
ΔE^*	36.62±0.58[a]	7.55±0.14[b]

[†]Values are given as mean ± S.E. from triplicate determinations (n=3). Different superscript letters in the same row indicate significant differences ($p<0.05$).

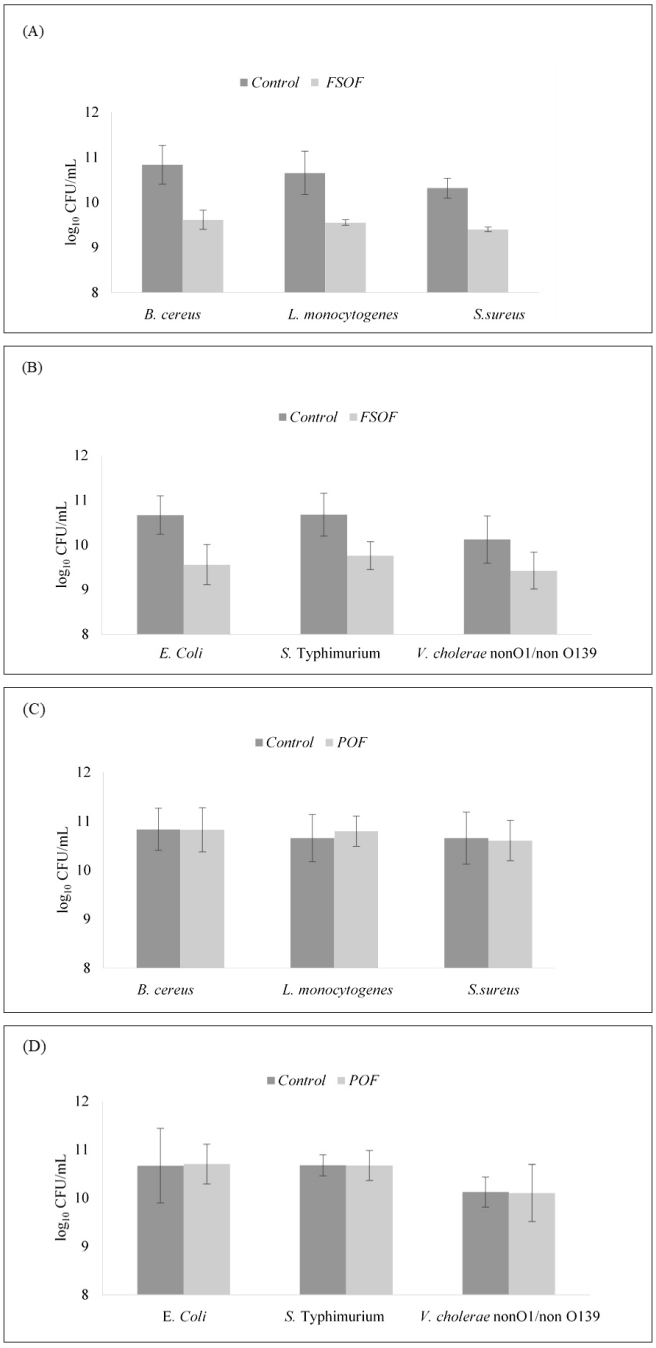

Figure 1. Antimicrobial activity of gelatin-based film emulsified with *R. tigerina* skin oil (A, B) and palm oil (C,D) against Gram-positive (A,C) and Gram-negative (B, D) food-borne pathogenic bacteria using suspension culture medium assay. FSOF: Frog skin oil emulsion film; POF: Palm oil emulsion film; Control: without film sample.

Impact of frog skin oil on characteristics of emulsion gelatin-based film and its antimicrobial activity

Characteristics of frog skin oil emulsion film (FSOF)

The film thickness is being the important criteria to decide for its application. The FSOF showed lower thickness than POF ($P<0.05$) (Table 2). The result might indicate the different capacity of the oil to disperse as oil droplet in gelatin-based films, which was governed by the lipid composition of the oil. Nilsuwan, Benjakul, and Prodpran (2016) and Tongnuanchan et al. (2016) also reported on the different

thickness of gelatin-based film samples, which influenced by the oil droplet size and oil dispersions. The smaller droplet size could provide the thinner oil.

The color of film is an indicator for consumer acceptability and marketability. Incorporation of FSO affected the color of film (FSOF). The higher lightness and yellowness with higher L* and b* values, respectively, could be obtained from FSOF, compared to those of POF (Table 1). On the other hand, the similar redness (a* value) was found from POF and FSOF (P>0.05). The total color difference (*ΔE* values) of FSOF and POF were significantly difference as shown in Table 2. The highest *ΔE* values were observed from FSOF (P< 0.05). Due to FSOS showed their characteristic more yellowish coloration than POF consequently provoked the increase in color variation (*ΔE*). This indicated the impact of yellow color of FSO used for preparing the film. This was in accordance with the variance color of film packaging contained with natural yellow of essential oils or lipids (Nisar, Wang, Yang, Tian, Iqbal, & Guo, 2018). The coloring pigments/ compounds and their contents in oil caused the color change in gelatin film (Karnjanapratum, Nilsuwan, Benjakul, & Sumpavapol, 2018). Mohsenabadi, Rajaei, Tabatabaei, and Mohsenifar (2018) reported that the increasing of yellowness of film related to essential oil incorporated.

Antimicrobial activity of emulsion-based film using suspension assay

Antimicrobial activity of FSOF and POF was examined against Gram-positive (*B. cereus*, *L.monocytogenes* and *S. aureusi*) and Gram-negative (*E.coli, S* Typhimurium and *V. cholerae* non O1/non O139) food-borne pathogenic bacteria were shown in Figure 1. The presence of FSOS in broth system of suspension assay inhibited the growth of all types of microorganism, compared with control. This indicated that antimicrobial content of FSO could be released and inhibited the bacterial growth. POF did not show the effectiveness to inhibit microorganisms, both Gram-positive and Gram-negative, indicating that POF had no antimcrobial properties against the tested bacteria. These results confirmed that FSO contained with antimicrobial compound, which could not be found in the control (palm oil).

Antimicrobial activity of emulsion-based film using disk-diffusion assay

Inhibition activity of emulsion-based film was examined using disk-diffusion assay. The result shown that the negative result (without clear zone) was presented in all samples (data not shown). Under the condition used, FSO could not be released from the film matrix and diffuse into hydrophilic phase of the MHA agar (Karnjanapratum, et al., 2018). When comparison to two methods used for studying antimicrobial properties (suspension assay and disk-diffusion assay), the contrast result was observed. Thus, the selection of suitable method for investigating antimicrobial properties of samples is necessary.

CONCLUSIONS

Oil extracted from Asian bullfrog skin (FSO) could be an alternative natural source of antimicrobial compounds that possessed the bacteria growth inhibition activity on both Gram-positive and Gram-negative bacteria. Different physical characteristics between FSOF and POF were obtained. Gelatin film solutions emulsified with FSO (FSOS) and extracted frog skin oil in film form (FSOF) showed on the effective antimicrobial activities but there was absence in palm oil both in emulsified solution and film forms (POS and POF, respectively). It was noted that disk-diffusion assay was appropriate to measure growth inhibition in emulsified solution rather than in gelatin-based film. Since it was less sensitive than the MIC broth method or needed the stronger antimicrobial concentration so that expressed the inhibition zone.

ACKNOWLEDGEMENTS

This work was financially supported by KMITL Research Fund under Research Seed Grant for New Lecturer to Dr. Supatra Karnjanapratum (Grant No. KREF186014).

REFERENCES

Alencar, É. N., Xavier-Júnior, F. H., Morais, A. R. V., Dantas, T. R. F., Dantas-Santos, N., Verissimo, L. M., Rehder, V. L. G., Chaves, G. M., Oliveira, A. G., & Egito, E. S. T. (2015). Chemical characterization and antimicrobial activity evaluation of natural oil nanostructured emulsions. *Journal of Nanoscience and Nanotechnology, 15*(1), 880-888.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology, 37*(8), 911-917.

Buntin, N., Chanthachum, S., & Hongpattarakere, T. (2008). Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. *Songklanakarin Journal of Science and Technology, 30*, 141-148.

Canillac, N., & Mourey, A. (2001). Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. *Food Microbiology, 18*(3), 261-268.

Daszak, P., Schloege, l. L., Louise, M., Cronin, A., Pokras, M., Smith, K., & Picco, A. (2006). *The global trade in amphibians: Summary interim report of a CCM study in report of the consortium for conservation medicine.* New York: Consortium for Conservation Medicine.

Gennadios, A., Weller, C. L., Hanna, M. A., & Froning, G. W. (1996). Mechanical and Barrier Properties of Egg Albumen Films. *Journal of Food Science, 61*(3), 585-589.

Goldbeck, J. C., Victoria, F. N., Motta, A., Savegnago, L., Jacob, R. G., Perin, G., Lenardão, E. J., & Padilha da Silva, W. (2014). Bioactivity and morphological changes of bacterial cells after exposure to 3-(p-chlorophenyl)thio citronellal. *LWT - Food Science and Technology, 59*(2), 813-819.

Kannan, P., Shanmugavadivu, B., Petchiammal, C., & Hopper, W. (2006). In vitro antimicrobial activity of Wrightia tinctoria leaf extracts against skin microorganisms. *Acta Botanica Hungarica, 48*(3-4), 323-329.

Karnjanapratum, S., Nilsuwan, K., Benjakul, S., & Sumpavapol, P. (2018). Oil from Asian bullfrog (Rana tigerina) skin: Antimicrobial activity and its application in emulsion gelatin-based film. In *Conference on Food and Applied Bioscience: FAB 2018.*). Chiangmai, Thailand: Chiang Mai University.

Klangmuang, P., & Sothornvit, R. (2016). Barrier properties, mechanical properties and antimicrobial activity of hydroxypropyl methylcellulose-based nanocomposite films incorporated with Thai essential oils. *Food Hydrocolloids, 61*, 609-616.

Mith, H., Duré, R., Delcenserie, V., Zhiri, A., Daube, G., Clinquart, A. (2014). Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. Food Science and Nutrition 2: 403-416).

Mohsenabadi, N., Rajaei, A., Tabatabaei, M., & Mohsenifar, A. (2018). Physical and antimicrobial properties of starch-carboxy methyl cellulose film containing rosemary essential oils encapsulated in chitosan nanogel. *Int J Biol Macromol, 112*, 148-155.

Munhuweyi, K., Caleb, O. J., Lennox, C. L., van Reenen, A. J., & Opara, U. L. (2017). In vitro and in vivo antifungal activity of chitosan-essential oils against pomegranate fruit pathogens. *Postharvest Biology and Technology, 129*, 9-22.

Nilsuwan, K., Benjakul, S., & Prodpran, T. (2016). Influence of palm oil and glycerol on properties of fish skin gelatin-based films. *J Food Sci Technol, 53*(6), 2715-2724.

Nisar, T., Wang, Z. C., Yang, X., Tian, Y., Iqbal, M., & Guo, Y. (2018). Characterization of citrus pectin films integrated with clove bud essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. *Int J Biol Macromol, 106*, 670-680.

Oduntan, O., O., S., J. A. , & Jenyo-Oni, A. (2012). Comparison of edible frog (Ranaesculenta) and other bush meat types: Proximate composition, social status and acceptability. *Journal of Environmental Research and Management, 3*(7), 124-128.

Pariyanonth, P., & Daorerk, V. (1994). Frog farming in Thailand. In K. P. P. Nambiar & T. Singh (Eds.). In *INFOFISH-AQUATECH ´94 International Conference on Aquaculture*, (pp. 126-130). Colombo, Sri Lanka.

Tongnuanchan, P., Benjakul, S., & Prodpran, T. (2014). Comparative studies on properties and antioxidative activity of fish skin gelatin films incorporated with essential oils from various sources. *International Aquatic Research, 6*(2), 62.

Tongnuanchan, P., Benjakul, S., Prodpran, T., Pisuchpen, S., & Osako, K. (2016). Mechanical, thermal and heat sealing properties of fish skin gelatin film containing palm oil and basil essential oil with different surfactants. *Food Hydrocolloids, 56*, 93-107.

Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols, 3*(163-175).

Wongtavatchai, J., Rungsipipat, A., Chumkaeo, A., & Surachetpong, W. (2003). Common diseases of farmed frogs Rana tigerina in thailand. In *In Proceedings 11th International Symposium of the World Association of Veterinary Laboratory Diagnosticians and OIE Seminar on Biotechnology*). Bangkok, Thailand: Thai Veterinary Medical Association.

Zhang, Y., Liu, X., Wang, Y., Jiang, P., Quek, S.Y. (2016). Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. Food Control 59: 282-289.

Zinoviadou, K.G., Koutsoumanis, C.P., Biliaderis, C.G. 2009. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. Meat Science, 82, 338-345.