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Comparison Study on Antimicrobial Activity and Antimicrobial Agent Release of Virgin Coconut Oil (VCO) and Lauric Acid Modified Pullulan/Starch Based Films

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Abstract: Antimicrobial (AM) packaging is a type of packaging that can reduce or inhibit the growth of spoilage microorganisms by the release of AM agent to the packaged food, and thus extending the shelf life of food. Virgin coconut oil (VCO) is one of the AM agent, which is easily available in Malaysia and have the potential to be applied in food packaging due to its AM, antiviral and antifungal properties. The AM property of VCO majorly contributed by its free fatty acids and their monoglycerides, especially lauric acid and monolaurin. Thus, the aim of this study is to evaluate the AM activity of VCO and lauric acid as AM agent. The VCO and lauric acid modified pullulan/starch-based films were prepared by casting technique. The AM activity of VCO and lauric acid against Escherichia coli (E.coli) were tested by agar diffusion test. The inhibition zone was observed on disks containing lauric acid modified pullulan/starch-based film solution only. It indicates that only lauric acid had inhibitory effect on E.coli. Lauric acid modified pullulan/starch-based film at 9:1 ratio had the highest inhibition zone diameter (1.7 mm) due to the presence of the highest concentration of lauric acid, which was 3.6 mg/mL. The release of AM agent from modified pullulan/starch-based film was evaluated by liquid culture test. The result showed that lauric acid modified pullulan/starch-based film had longer AM agent release period than VCO, particularly at 9:1 ratio. It had the longest duration of AM agent release which was 13 hours. Consequently, lauric acid is able to inhibit the growth of bacteria for a longer time than VCO. It can be concluded that lauric acid is more effective as AM agent compared to VCO. Additionally, lauric acid modified pullulan/starch-based film at 9:1 ratio is the most effective formulation for the AM film.

Keywords: Antimicrobial packaging, Virgin coconut oil, Lauric acid,

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1. Introduction

Nowadays, a great attention has been paid on the safety issues of the packaged food. Food is packaged for the purpose of preservation and storage for a long period of time [1]. The quality of packaged foodstuff is highly depending on the types of packaging and the packaging material properties. As such, active food packaging is developed to extend the shelf life of food products and to improve the food products quality. Active packaging is packaging in which subsidiary components have been deliberately added in or on the package headspace or packaging material to improve the performance of the package system [2]. Antimicrobial (AM) packaging is one of the active packaging, which is able to reduce or inhibit the growth of bacteria, thus preventing the microbial contamination of food. Virgin coconut oil (VCO) is one of the example of AM agent. The AM property of VCO is majorly due to its free medium chain fatty acids (MCFAs) and their monoglycerides, especially lauric acid and monolaurin.

Apart from emphasis on food safety and quality of food packaging materials, the environmental impact of the packaging materials are concerning too. Normally, the packaging materials are derived from petroleum products that can cause the problem in waste disposal [3]. Thus, biodegradable food packaging materials are highly recommended instead of non-biodegradable materials for the purpose of sustainability and environmental protection, such as starch and pullulan. Biodegradable food packaging material is biopolymer in which at least one of the step in the degradation process is carried out by the metabolism of naturally occurring organisms [4]. Biodegradation enables decomposition of the plastics with no toxic or environmentally harmful residues under appropriate conditions of temperature, oxygen availability and moisture. Starch is one of the biopolymer that occurring naturally in a variety of plant sources such as corn, rice, potato and wheat [5]. Starch is able to interact with many components and additives of the food [6]. However, starch has poor moisture barrier property and poorer mechanical properties than non-biodegradable plastic films that had been used in food packaging industries [5]. This can be improved by the mixing of starch with pullulan. Pullulan has considerable mechanical strength and barrier properties against carbon dioxide and oxygen [7].

Thus, this study was done to investigate the AM efficiency of VCO and lauric acid in pullulan/starch-based film against E.coli as food packaging material, which are biodegradable and having AM properties.

1.1 Active Food Packaging

Advances in science and technology have catalyzed the development of food packaging technology. The food packaging technology is divided into many categories, such as active food packaging, intelligent food packaging, edible food packaging and biodegradable packaging. In active food packaging, the shelf life of processed food can be extended by delivering AM agents to food surface [8]. Currently, incorporation of natural AM agent is gaining a wide popularity in food packaging due to its biodegradability. This section review and focus on the AM properties of VCO and lauric acid in food packaging application

Active packaging is a type of food packaging that can sense the external or internal change and respond to it by changing its own attributes or characteristics [9]. Packaging materials incorporating active ingredients and compounds can provide several functions that do not found in traditional food packaging systems [10]. Active food packaging is effective in preventing quality loss and extending in packaged food products through the control of microbial activity in packaged food. Active packaging can be categorized into two main systems, which are releasing and adsorbing system [11]. The example of releasing systems are carbon dioxide emitters, ethanol emitters, odor releasers and AM agent releasers, while for the absorbing systems are such as oxygen absorbers, moisture absorbers and odor absorbers [11]

AM packaging is one of the active packaging. The AM packaging is able to reduce or inhibit the growth of spoilage microorganisms by the releases of AM agent to the packaged food and thus reduce the occurring of microbiological contamination. The AM agents inhibit or reduce spoilage microorganism by inhibit some essential metabolism activity, reproductive genetic pathway or alter cell wall / membrane structure of microorganisms. For instance, lysozyme can destroy cell wall of microorganism to inhibit or reduce microorganism's growth [12].

AM packaging is divided into several systems such as dispersing bioactive agents in the packaging, adding a sachet into the food package, utilizing AM agents that containing film-forming properties, or coating bioactive agents on the surface of packaging materials [13]. Incorporating of AM agents into food packaging allow slow release of AM substances onto the food surface.

The release of AM agents to the packaged food is divide into two systems, which are migrating system and non-migrating system. In the migrating system, AM agent is volatile and thus the AM agent will be release from packaging film to the headspace and finally onto the surface of packaged food. In the non-migrating system, the non-volatile AM agent requires direct contact of packaging film with food to allow it to diffuse to the packaged food [14]. Many researchers are attracted to natural AM agents in food packaging as the replacement for synthetic AM agents. This is because the great consumers demand on natural food preservation from microbial contamination instead of synthetic chemical preservative [15]. Natural AM agents are categorized by their origin, which are derived from animal origin (eg: lactoferrin, lysozyme); plant essential oils (eg: clove, VCO, cinnamon, basil, rosemary); naturally occurring polymers (chitosan, UV/excimer laser irradiated nylon); and microbial origin (eg: natamycin, nisin) [14]. The natural AM agents that derived from essential oils and herbs are considered as safe food additive and are "Generally Recognized As Safe" (GRAS) by the American Food and Drug Administration [16]

1.2 Virgin Coconut Oil and Lauric acid as Antimicrobial Agent

Virgin coconut oil (VCO) is the oil that obtained from the mature kernel of the fresh coconut by natural or mechanical method, with or without using of heat and without undergoing any chemical process. VCO extraction methods are wet extraction, chilling, freezing and thawing techniques, fermentation technique and enzymatic techniques. VCO is colourless, tasteless and with aroma as fresh coconut. VCO contains a large amount of medium chain fatty acids (MCFAs) such as capric acid, lauric acid, and caproic acid [17], which plays a role for its AM activity. Lauric acid has better AM property compared to myristic acid, capric acid and caprylic acid. Monogylcerides and free fatty acids disrupt the cell membrane of lipid bilayer to inactive food spoilage microorganisms [18]. Lauric acid is the most abundant fatty acid present in VCO, which is about 45.0-56.0%. VCO has AM property against both gram negative and positive bacteria [19]. Lauric acid and monolaurin. Silalahi and her partners in 2014 [18], reported that the combination of monoglycerides and free fatty acids has synergistic effect to bacteria innibition. It was found that 10.0 % (v/v) of VCO may extend the lag phase of S.aureus CH1 to about 240 minutes [20]. Furthermore, VCO possess in-vitro AM properties against Candida sp.by agar-well diffusion technique, thus it can act as antifungal [21].

Lauric acid is a saturated medium chain fatty acid that contains 12 carbon backbone (C 12:0). It is a weak acid. In VCO, it is in the form of triglycerides; whereas, in human body, the triglycerides will be converted into lauric acid and monoglycerides which containing AM activity [22]. Lauric acid is also known as dodecanoic acid or dodecyclic acid. It is normally found in animal and plant oils and fats, especially in palm kernel oil and coconut oil. Lauric acid has high hydrophobicity which can alter the hydrogen bonding and dipole-dipole interaction between acyl chains. This disrupt the glycerophospholipid molecules inside the membrane [20].

Lauric acid incorporated into chitosan/starch based film inhibits both E. coli and B. subtilis, and the increase in lauric acid concentration can increase the AM activity against E. coli through agar diffusion

test and liquid culture test [23]. Another researcher found that 5 % of lauric acid could inhibit the growth of B. cereus, Staph. aureus, E. coli and Salmonella typhimurium through well diffusion test [24]. It was fpound that lauric acid could inhibit S. pyogenes with minimal inhibitory concentration (MIC) of 125 µg/mL by using agar diffusion test [25].

2. Materials and Methods

In this study, the VCO and lauric acid incorporated pullulan/starch based-film was produced by casting method. The tests on the AM activity of the VCO and lauric acid against E. coli was investigated through agar diffusion test. While the release of AM agents was studied by liquid culture test by using ultra-violet spectrophotometer (UV-VIS).

2.1 Film Preparation

VCO was purchased from Institute Bioproduct Development, UTM (Malaysia), lauric acid was purchased from Fluka Chemika (Malaysia), starch and glycerol were purchased from Emory (Malaysia), pullulan was brought from Japan, nutrient agar and nutrient broth were purchased from Merk (Germany).

2.2 Methods

5 g of starch was dissolved in 100 ml of distilled water. Glycerol was added to the starch solution as plasticizer. The amount of glycerol added is half amount of the starch used. The starch solution was mixed gently by using magnetic stirrer with the addition of slow heating. When the solution reaches 80-86 ℃, the solution become clear and the stirring process was stopped. After that, 5 g of pullulan was dissolved in 100 ml of distilled water with gently stirring. The solution of starch and pullulan were mixed in different ratio according to the blend formulations [9:1, 8:2, 7:3 starch/pullulan (w/w)]. Then, 8.0 % of VCO/lauric acid (based on the mass of starch used) was added to the solution. The solution was gently stirred with slow heating until to the solution mixed completely. A 5 ml of film making solution was pipetted and spread evenly into a petri dish with an area of 90 mm x 15 mm. After that, it was air drying at room temperature overnight. The films produced were labelled as shown in the Table 1, 2 and 3.

Table 1: **The percent composition of starch and pullulan mixture mixed with VCO**

Sample	Starch (wt%)	Pullulan (wt%)	VCO (%)
A1	90	10	8
A2	80	20	8
A3	70	30	8

Table 2: **The percent composition of starch and pullulan mixture mixed with lauric acid**

Sample	Starch (wt%)	Pullulan (wt%)	Lauric acid (%)
B1	90	10	8
B2	80	20	8
B3	70	30	8

Table 3: **The percent composition of starch and pullulan mixture**

Sample	Starch (wt%)	Pullulan (wt%)
C1	100	0
C2	90	10
C3	80	20
C4	70	30

2.3 Agar Diffusion Test

Agar diffusion test is used to evaluate the AM activity of sample against E. coli. The filter paper (grade A1) was cut into disk (diameter = 6mm). 100 μL of inoculum solution was pipetted onto the agar plate surface and spread evenly by using a glass spreader. The agar plate was air dried in biosafety cabinet. The cut disk was immersed into sample solution (from the film preparation) and placed on top of agar. After the cut disk attached on the top of agar plate, the plate was sealed and inverted. The disk containing starch and pullulan/starch films acts as control. Each type of films was to be done in triplicate. Duplicate agar plates were prepared for each type of film. The plates were incubated in anincubator at 37 ℃ for 24 hours (h). After 24 h, the agar plate was visually observed and the inhibition diameter was measured.

2.3 Liquid Culture Test

The main objective of liquid culture test was to evaluate the AM agents (VCO and lauric acid) release against *E.coli* [26-27]. It also indicates the AM activity of VCO and lauric acid. Optical density is one of the measure to indicate the concentration of bacteria in liquid culture [28]. It measures the amount biomass of bacteria present in a suspension.

Each type of sample film was cut into a square (1 cm x 1 cm). Three cut sample films were immersed into 20 ml of nutrient broth in a universal bottle. 200 μL of inoculum was inoculated into the sample containing nutrient broth. The universal bottles were placed in orbital shaker and rotated at 200 rpm at 37 ℃. During incubation, the cultures were sampled periodically (0h, 2h, 5h, 22h, and 24h) to get the bacteria growth profile. The optical density of sample was determined by using ultra-violet spectrophotometer (UV-VIS) at wavelength, λ = 600 nm. The test was done in triplicate.

3. Results and Discussion

The results and discussions were divided into three section ;antimicrobial activity of VCO and lauric acid modified pullulan/starch-based film by agar diffusion test, antimicrobial agent release from VCO and lauric acid modified pullulan/starch-based film and relationship between the agar diffusion test and liquid culture test.

3.1 Antimicrobial activity of VCO and lauric acid modified pullulan/starch-based film by agar diffusion test

Through the agar diffusion test, the antimicrobial (AM) activity of VCO and lauric acid modified pullulan/starch-based film against *E.coli* were investigated. The result of the agar diffusion test for the inhibition of *E.coli* growth on the VCO and lauric acid modified pullulan/starch-based film is shown in Figure 1.

From the Figure 1, there was no inhibition zone on *E.coli* can be observed from starch and starch/pullulan blend sample. This indicates that there was no AM activity from these samples against *E.coli*. Starch and starch/pullulan control films did not have AM activity because of the absence of AM agent, such as lauric acid or VCO. However, pullulan potentially inhibit the fungal growth. Pullulan is categorized as un-assimilable organic compound. According to liu and his co-workers [29], assimilable organic compound means the low-molecular-weight dissolved organic carbon molecules which can be easily assimilated by bacteria. Therefore, pullulan is difficult to be assimilated by bacteria. Although no AM agent found in pullulan, this characteristic may retard the bacteria or fungi growth which resulting to food spoilage [30]. Thus, pullulan is able to extend the shelf life of packaged food.

From the result, it can be observed that VCO modified pullulan/starch-based film did not possess AM activity against *E.coli*. It is possibly due to the presence of small amount of free fatty acids in VCO and it does not contain monolaurin. It is highlighted that the AM activity of VCO depends on the

presence of medium chain free fatty acid and monoglycerides, particularly lauric acid and monolaurin. These two components can be released by hydrolysis of triglycerides in VCO. According to study done by Silalahi and her co-workers in 2014 [18], reported that VCO did not demonstrate AM activity because of the insufficient of free fatty acid and absence of monolaurin. In addition, the VCO used was not hydrolyzed. Without hydrolysis, lauric acid and monoglycerides are in the form of triglycerides, which do not have AM activity. Based on the previous study, it showed that hydrolyzed VCO had AM activity but unhydrolyzed VCO did not have the AM activity [20]. VCO did not show AM activity may also due to the lipase activity inside the VCO is low, leading to low concentration of free fatty acid (lauric acid) release. According to the previous study which used the same origin of VCO, it was found that the free fatty acid as lauric acid in VCO and hydrolyzed VCO were 0.14 % and 17.40 % respectively [31]. It proved that the lauric acid content in VCO was greatly lower than hydrolyzed VCO. Furthermore, VCO had complex chemical structure and low solubility in water, causing it had difficulty in dissolving onto the surface of agar from the sample film to inhibit bacteria growth. Based on the research done by Chiaw and her partners in 2010 [17], it was found that VCO did not exhibit AM activity against *E. coli* because of the complex structure of VCO and the lauric acid released by naturally occurring enzyme lipase did not have sufficient AM activity to inhibit bacteria growth at low concentration. Thus, VCO did not have adequate ability to inhibit the growth of *E.coli*.

From Figure 1, it can be observed that lauric acid modified pullulan/starch-based film possess AM activity against *E.coli* by agar diffusion test. The inhibition of *E.coli* was expressed by the inhibition zone diameter. The inhibition zone diameter produced by lauric acid modified pullulan/starch-based film increased when the concentration of lauric acid increased. B1 had highest amount of lauric acid (3.6 mg/ml), followed by B2 (3.2 mg/ml) and B3 (2.8 mg/ml). Lauric acid is a type of fatty acid which has AM agent. As a result, it led to the largest inhibition zone diameter (1.7 mm) in B1 while B3 had the smallest inhibition zone diameter (0.4 mm). According to the Salleh and her co-workers' research in 2014 [23], it was reported that the higher the amount of lauric acid, the better the inhibition effect towards bacteria.

Lauric acid might inhibit the growth of *E.coli* by inactivation of enzyme, denaturation of cell protein and disruption of cell membrane. According the previous study [22], showed that the undissociated lauric acid can easily penetrate the cell membrane of microbe cell and dissociate within the cell. As a result, it would interfere neutral pH of the cell cytoplasm, leading to damage of cell membrane and impaired of cellular energy production, inhibit the synthesis of macromolecules or even denaturation of DNA and protein. Furthermore, the energy production in the bacterial cell membrane can be influenced by free fatty acid through the oxidative phosphorilation and disruption of electron transport chain [18]. Lauric acid has high hydrophobicity which can alter the hydrogen bonding and dipole-dipole interaction between acyl chains. This disrupt the glycerophospholipid molecules inside the membrane as mentioned in the previous research [20]. Thus, it is believe that lauric acid has the ability to inhibit the growth of *E.coli* and the AM activity increases with the lauric acid concentration.

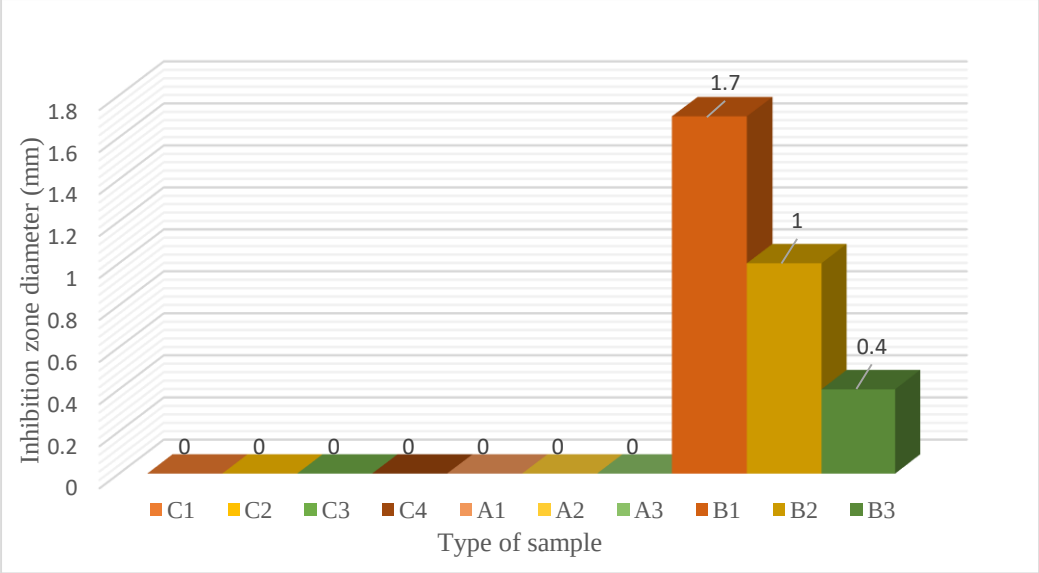

Figure 1: Antimicrobial activity (inhibition zone diameter) of VCO and lauric acid modified pullulan/starch-based film against *E.coli*

3.2 Antimicrobial agent release from VCO modified pullulan/starch-based film

Figure 2 shows the graph of optical density (600 nm) versus incubation time for starch, pullulan/starch-based and VCO modified pullulan/starch-based films against E.coli. From the first 5 h, the optical density of the pullulan/starch-based films were almost the same with starch film (control). At 24 h incubation time, optical density of C1 equals to 1.089, while the optical density of pullulan/starch-based film were 1.004, 1.006 and 1.053 for C2, C3 and C4 respectively. There was no AM agents released in pullulan/starch-based films because the pullulan/starch-based film did not significantly reduce the concentration of E.coli. Pullulan does not has antibacterial activity [32].

For the VCO modified pullulan/strach-based films, at the first 5 h, the E.coli concentration for the VCO films and control films were almost the same, indicating no AM agent release. From the incubation hours 5 to 22 h, there was a small amount of AM agent release because there was reduction of bacteria amount in A1 and A3 compared to control film. VCO is AM agent which exhibits inhibitory effect in the presence of free fatty acid and monoglycerides [18]. For example lauric acid and monolaurin, which play a major role in VCO AM activity. The free fatty acids and monoglycerides released from VCO by naturally occurring lipase present in the VCO. Hence, VCO released produces free fatty acid acids and monoglycerides to retard the growth of E.coli. However, at 24 h, the amount of E.coli in A1, A2 and A3 did not have much different with C1, C2, C3 and C4. This indicates there was no AM agent release in incubation time, 24 h. On the other words, it may have VCO release, but the amount of VCO release is not sufficient for it to act as AM agent to inhibit E.coli growth. According to study done by Tangwatcharin and her partners in 2012 [20], it was found that VCO could not inhibit bacteria growth, but VCO may extend the lag phase of bacteria. A1 could release AM agent from 13 h until 22 h of incubation time. A3 had the longest AM agent release time, which was began from 11 h to 22 h of incubation time, 11 h of AM agent release. Thus, it shows that VCO modified pullulan/starch-based film can release AM agent but the AM agent release period no longer than 11 h.

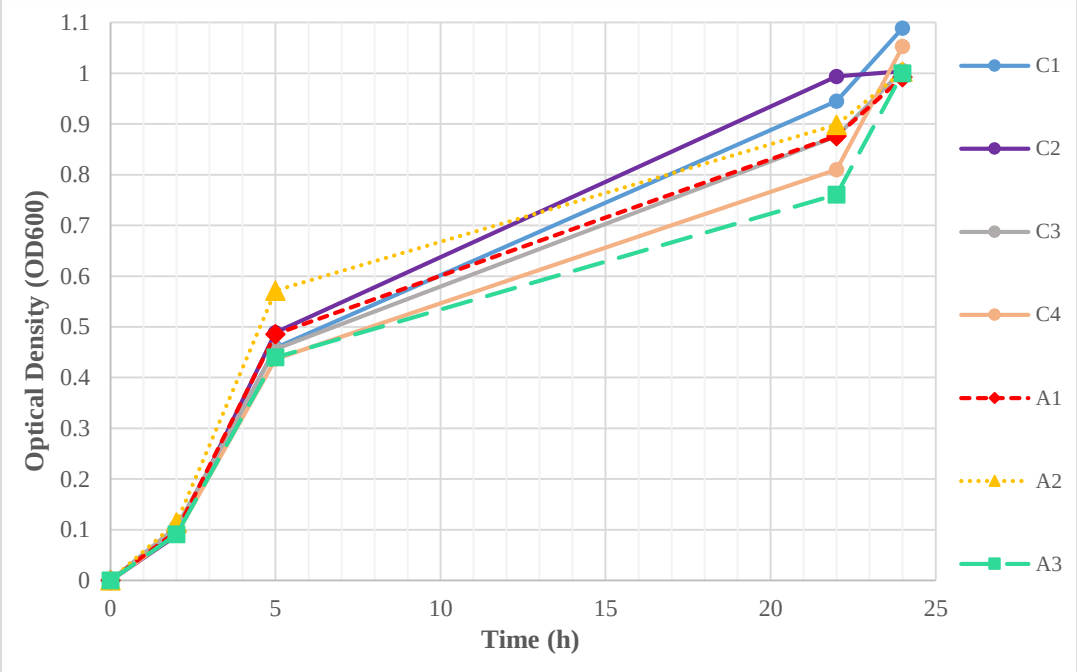

Figure 2: Growth profile of E.coli of starch, pullulan/starch-based and VCO modified pullulan/starch-based films in liquid culture test

3.3 Antimicrobial agent release from lauric acid modified pullulan/starch-based film

Figure 3 illustrates the graph of optical density (600 nm) versus incubation time for starch, pullulan/starch-based and lauric acid pullulan/starch-based films against E.coli. From the first 5 h, the optical density of films were almost the same with control films. After that, there were differences in term of optical density between these films, meaning that AM agent started to be released. At 24 h of incubation time, optical density of C1 equals to 1.089, while the optical density of other films were 1.004, 1.006, 1.053, 0.853, 0.892 and 0.946 for C2, C3, C4, B1, B2 and B3 respectively. B1 had the highest deviation from C1 with a percentage of 21.67 %, followed by B2 (18.09 %) and B3 (13.13 %). The higher the amount AM agent release, the higher the AM activity, the lower the optical density. Optical density indicates the concentration of bacteria present. B1 possessed the highest AM activity, which meaning that B1 has the highest concentration of AM agent release. The AM agent release begin from 11 h and 14 h of incubation time for B1 and B2 respectively. B3 shows AM agents released from time 15 h. Thus, B1 had the longest AM agent release duration, which was 13 h. The AM agent in the lauric acid modified pullulan/starch-based film is lauric acid. The concentration of lauric acid present in film is 3.6 mg/mL, 3.2 mg/mL and 2.8 mg/mL for B1, B2 and B3 respectively.

Lauric acid can be released to the nutrient broth by following mechanisms. Firstly, broth solution penetrates and diffuses into the lauric acid pullulan/starch-based film, resulting swelling of film. This leads to the knits of the polymeric network of film become wider, and thus allowing the lauric acid to diffuse from the matrix of film into the nutrient broth solution, until thermodynamic equilibrium reached [33]. The higher amount of lauric acid present in the film requires higher concentration of lauric acid release into the solution in order to reach thermodynamic equilibrium. As such, the higher the concentration of lauric acid present in film, the higher the amount of AM agent release, the stronger the bacteria inhibition effect. 3.4

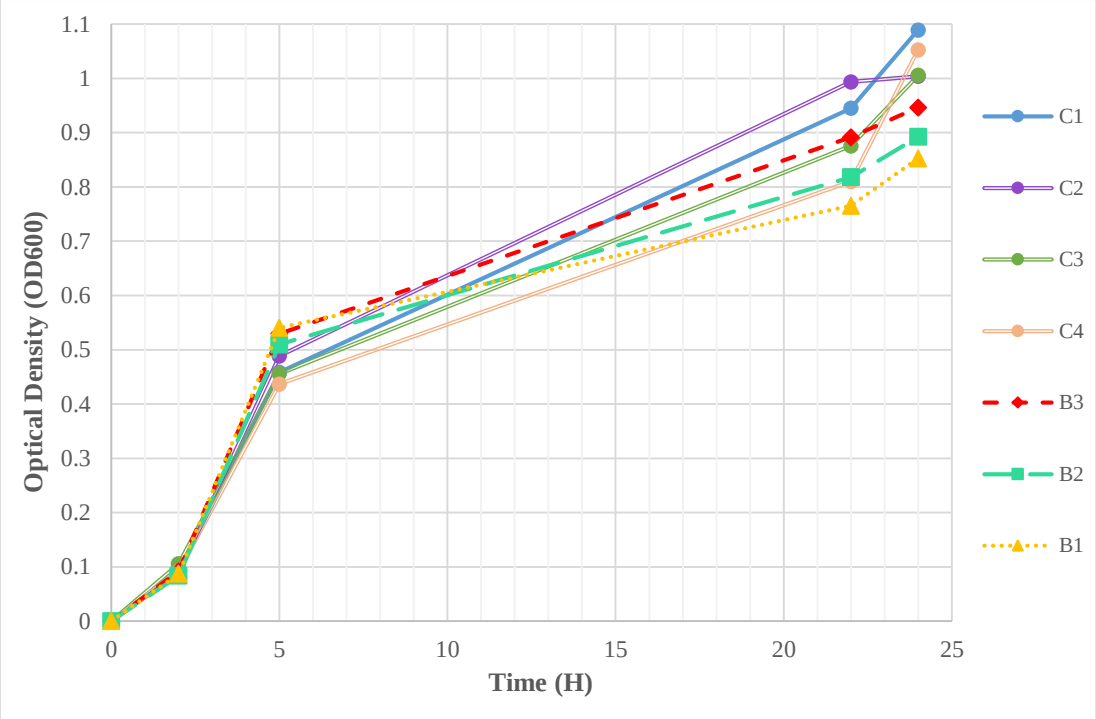

Figure 3: Growth profile of E.coli for starch, pullulan/starch-based and lauric acid modified pullulan/starch-based films in liquid culture test

3.4 Relationship between the agar diffusion test and liquid culture test

Figure 4 shows the inhibitory effect of VCO and lauric acid modified pullulan/starch-based films in agar diffusion test and liquid culture test. Liquid culture test also can be used to show the AM activity of VCO and lauric acid against E.coli. Figure 4 demonstrates that VCO exhibit the AM activity only in liquid culture test. However, not significant inhibitory effect of VCO observed in liquid culture test. Interestingly, lauric acid demonstrated inhibitory effect in both test. Liquid culture test showed better inhibitory effect compared to agar diffusion test due to the agitation in liquid medium used, which increased the diffusion of AM agent into liquid medium and the mobility of bacteria [23].

As shown in Figure 4, Lauric acid modified pullulan/starch-based films had greater AM activity than VCO modified pullulan/starch-based film. Lauric acid modified pullulan/starch-based film at ratio 9:1 showed the highest AM activity because it had largest value of inhibition zone diameter (1.7mm) and reduction optical density (15.04%). This may due to AM agent show lower AM activity in gram negative bacteria, E.coli. Gram negative bacteria has protective outer membrane surrounding both cell wall and peptidoglycan layer compared to gram positive bacteria [5]. Further, this increased the difficulties to retard the growth of bacteria. Based on recent study [20], it showed that lauric acid exhibited AM activity, whereas VCO did not have AM activity. The AM activity increased with the concentration of lauric acid. This is in agreement with the results obtained.

For the liquid culture test, it also demonstrated that lauric acid modified pullulan/starch-based film can release AM agent for a longer time than VCO modified pullulan/starch-based film, thus allowing a longer period of bacteria inhibition. B1 had the longest AM agent release period, which was 13 h. Therefore, lauric acid modified pullulan/starch-based films demonstrate better AM activity and AM agent release than VCO modified pullulan/starch-based films, and B1 is the most effective film formulation for the inhibition of E.coli growth with longest inhibition period among all of the sample films.

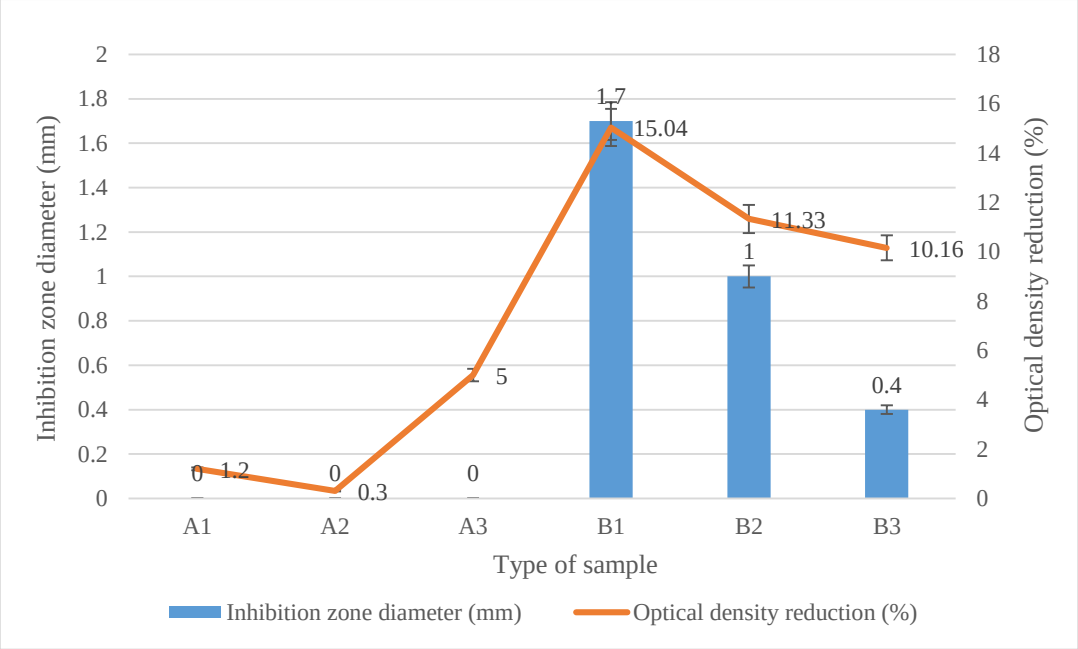

Figure 4: Relationship between the agar diffusion test and liquid culture test for 24 h incubation hour

4. Conclusion

The antimicrobial (AM) efficiency of VCO and lauric acid in pullulan/starch-based film against E.coli were investigated. The lauric acid modified pullulan/starch-based film exhibited greater AM activity than VCO modified pullulan/starch-based film. VCO did not demonstrate AM activity in agar diffusion test due to the presence of small amount of free fatty acids and monoglycerides, particularly lauric acid and monolaurin. Free fatty acids and monoglycerides were released by the hydrolysis action of naturally occurring lipase. The small amount of free fatty acids and monoglycerides did not have sufficient AM strength to retard the growth of E-COLI. B1 had the largest AM activity due to the presence of highest concentration of lauric acid in pullulan/starch-based film, which was 3.6 mg/mL.The higher the concentration of lauric acid contained in the film, the largest the inhibitory effect. Lauric acid might inhibit the growth of E.coli by inactivation of enzyme, denaturation of cell protein and disruption of cell membrane.

The liquid culture test shows that lauric acid modified pullulan/starch-based film had longer AM agent release period than VCO, especially B1, which had 13 h of AM agent release within 24 h. VCO containing films only exhibited a short period of AM agent release. Based on to all of the results obtained, that lauric acid has more potential to act as AM agent than VCO in food packaging application. Starch/pullulan blend film at ratio 9:1 incorporated with lauric acid showed the most effective AM activity and AM agent release compared to other formulations.

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References

[1] Galic, K., Scetar, M., & Kurek, M. (2011). The benefits of processing and packaging. Trends in Food Science and Technology, 22(2-3), 127–137.

[2] Robertson, G. L. (2012). Food Packaging: Principles and Practice (Third Edit). United States: CRC Press.

[3] Avella, M., De Vlieger, J. J., Errico, M. E., Fischer, S., Vacca, P., & Volpe, M. G. (2005). Biodegradable starch/clay nanocomposite films for food packaging applications. Food Chemistry, 93(3), 467–474.

[4] Sorrentino, A., Gorrasi, G., & Vittoria, V. (2007). Potential perspectives of bio-nanocomposites for food packaging applications. Trends in Food Science & Technology, 18(2), 84–95.

[5] Salleh, E., & Muhamad, I. I. (2010). Starch-based antimicrobial films incorporated with lauric acid and chitosan. AIP Conference Proceedings, 1217, 432–436.

[6] Famá, L., Rojas, A. M., Goyanes, S., & Gerschenson, L. (2005). Mechanical properties of tapioca-starch edible films containing sorbates. LWT - Food Science and Technology, 38(6), 631–639.

[7] Farris, S., Unalan, I. U., Introzzi, L., Fuentes-Alventosa, J. M., & Cozzolino, C. A. (2014). Pullulan-based films and coatings for food packaging: Present applications, emerging opportunities, and future challenges. Journal of Applied Polymer Science, 131(13).

[8] Salleh, E., & Muhamad, I. (2007). Mechanical properties and antimicrobial analysis of antimicrobial starch-based film. International Conference on Advancement of Materials and Nanotechnology, 1–14

[9] Brody, A. L., Strupinsky, E. R., & Kline, L. R. (2001). Active Packaging for Food Applications. United States: CRC Press.

[10] Carrizo, D., Taborda, G., Nerín, C., & Bosetti, O. (2015). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. Innovative Food Science & Emerging Technologies, 33, 534–541.

[11] Pereira de Abreu, D. A., Cruz, J. M., & Paseiro Losada, P. (2012). Active and Intelligent Packaging for the Food Industry. Food Reviews International, 28(2), 146–187.

[12] La Storia, A. (2008). Development and Application of Antimicrobial Food Packaging. Università degli Studi di Napoli Federico II.

[13] Coma, V. (2008). Bioactive packaging technologies for extended shelf life of meat-based products. Meat Science, 78(1-2), 90–103.

[14] Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J., & Bigger, S. W. (2011). Essential Oils and Their Principal Constituents as Antimicrobial Agents for Synthetic Packaging Films. Journal of Food Science, 76(9).

[15] Lucera, A., Costa, C., Conte, A., & Del Nobile, M. A. (2012). Food applications of natural antimicrobial compounds. Frontiers in Microbiology, 3, 287.

[16] Matan, N., Rimkeeree, H., Mawson, A. J., Chompreeda, P., Haruthaithanasan, V., & Parker, M. (2006). Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. International Journal of Food Microbiology, 107(2), 180–5.

[17] Chiaw, M. S., Hip, S. Y., & Lai, C. M. (2010). Commercial virgin cooconut oil: assessment of antimicrobial potential. Asian Journal Of Food and Agro-Industry, 3(06), 567–579.

[18] Silalahi, J., Yademetripermata, & Putra, E. de L. (2014). Antibacterial activity of hydrolyzed virgin coconut oil. Asian Journal of Pharmaceutical and Clinical Research, 7(SUPPL. 2), 90–94.

[19] Satheesh, N., & Prasad, N. B. L. (2014). Production of virgin coconut oil by induced fermentation with Lactobacillus plantarum NDRI strain 184. Croatian Journal of Food Technology, Biotechnology and Nutrition, 9(1-2), 37–42.

[20] Tangwatcharin, P., & Khopaibool, P. (2012). Activity of virgin coconut oil, lauric acid or monolaurin in combination with lactic acid against Staphylococcus aureus. Southeast Asian Journal of Tropical Medicine and Public Health, 43(4), 969–985.

[21] Ogbolu, D. O., Oni, A. a., Daini, O. a., & Oloko, A. P. (2007). In Vitro Antimicrobial Properties of Coconut Oil on Candida Species in Ibadan, Nigeria. Journal of Medicinal Food, 10(2), 384–387.

[22] Elysa, Harahap, U., & Silalahi, J. (2014). Antibacterial activity of enzymatic hydrolysis of virgin coconut oil against Salmonella. International Journal of PharmTech Research, 6(2), 589–599.

[23] Salleh, E., Muhammad, I. I., & Pahlawi, Q. A. (2014). Spectrum Activity and Lauric Acid Release Behaviour of Antimicrobial Starch-based Film. Procedia Chemistry, 9, 11–22.

[24] Nitbani, F. O., Jumina, Siswanta, D., & Solikhah, E. N. (2016). Isolation and Antibacterial Activity Test of Lauric Acid from Crude Coconut Oil (Cocos nucifera L.). Procedia Chemistry, 18(Mcls 2015), 132–140.

[25] Batovska, D. I., Todorova, I. T., Tsvetkova, I. V., & Najdenski, H. M. (2009). Antibacterial study of the medium chain fatty acids and their 1-monoglycerides: Individual effects and synergistic relationships. Polish Journal of Microbiology, 58(1), 43–47.

[26] Hanušová, K., Dobiáš, J., & Klaudisová, K. (2009). Effect of packaging films releasing antimicrobial agents on stability of food products. Czech Journal of Food Sciences, 27(SPEC. ISS.), 347–349.

[27] Dawson, P. L., Carl, G. D., Acton, J. C., & Han, I. Y. (2002). Effect of lauric acid and nisin-impregnated soy-based films on the growth of Listeria monocytogenes on turkey bologna. Poultry Science, 81(5), 721–726.

[28] Myers, J. A., Curtis, B. S., Curtis, W. R., Svanberg, S., Swanson, N., Billard, B., … Kuo, T.-C. (2013). Improving accuracy of cell and chromophore concentration measurements using optical density. BMC Biophysics, 6(1), 4.

[29] Liu, X., Wang, J., Liu, T., Kong, W., He, X., Jin, Y., & Zhang, B. (2015). Effects of assimilable organic carbon and free chlorine on bacterial growth in drinking water. PloS One, 10(6), e0128825.

[30] Singh, R. S., Saini, G. K., & Kennedy, J. F. (2008). Pullulan: Microbial sources, production and applications. Carbohydrate Polymers, 73(4), 515–531.

[31] Shanmugam, S. A. (2016). Comparison on Antibacterial and Physicochemical Properties of Virgin Coconut Oil, Hydrolyzed Virgin Coconut Oil and Virgin Olive Oil. UTHM.

[32] Gniewosz, M., Kraśniewska, K., Woreta, M., & Kosakowska, O. (2013). Antimicrobial activity of a pullulan-caraway essential oil coating on reduction of food microorganisms and quality in fresh baby carrot. Journal of Food Science, 78(8).

[33] Mamat, S. N. H. M., Eraricar, S., & Salehudin, M. H. (2012). Kinetic and Release Rate of Lauric Acid from Starch-based Film in Food Stimulant. International Conference on Agricultural and Food Engineering for Life, 464–471.