Antibacterial Activities of Palmitic and Lauric Acids from Palm Kernel Oil for the Development of Food-Grade Disinfectants

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Abstract

Palmitic and lauric acids, derived from palm kernel oil, exhibit antibacterial activities that are less harmful to humans. This study aimed to evaluate the antibacterial activities and optimal concentrations of palmitic and lauric acids against *S. aureus* and *E. coli* using the agar well diffusion method, with the goal of developing a food-grade disinfectant. The results demonstrated that a ratio of 87.5:175 mg/ml of palmitic acid to lauric acid was the optimal mix for inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*. The average size of the inhibition zone was 10.40±0.57, 10.59±1.08, 10.57±0.40, 8.67±0.98, 6.38±0.29, and 10.98±0.59 mm. The MIC ratios (200, 62.5, 87.5 mg/ml) and MBC ratios (50, 62.5, 87.5 mg/ml) of palmitic acid combined with lauric acid were 800, 125, 175 mg/ml and 200, 250, 175 mg/ml, respectively. The study found that the combination of palmitic and lauric acids effectively inhibited *S. aureus* and *E. coli*. Furthermore, these acids could be used to disinfect food and reduce the reliance on chemical disinfectants in food manufacturing cleaning processes.

Keywords : Palmitic acid, Lauric acid, Disinfectant, *S. aureus*, *E. coli*

Introduction

The cleaning method is an essential part of food safety and sanitation control in food processing plants. The use of chemical disinfectants was widely applied and increased of use in food

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industry section [1]. Nowadays, we expose chemical contaminants of disinfectants that remain on food contact surface and have a negative impact on our long-term health. Then, the food grade disinfectants can help reduce using chemical disinfectant. Many researchers focus on fatty acid disinfectants, fatty acids (FA) there are 2 types of disinfecting properties: Palmitic acid (PA) and Lauric acid (LA). Palmitic acid (PA) and lauric acid (LA) are the main components of palm kernel oil (PKO), and PA and LA have antibacterial activity against pathogens such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Palm oil is a raw material used in the food and chemical industries for biofuel production due to the demand for vegetable oils in food and food products and the relatively low cost of palm oil. [2-7]. PA and LA is a product of the fatty acid synthase (FAS) complex, which is important for ensuing desaturation [8], PA increases cellular toxicity and the rate of cell damage due to genes responsible for fatty acid desaturation; damage to DNA, RNA, and proteins by reactive oxygen species (ROS), which are by-products of prokaryotes and eukaryotic cells, where cells are electron transport and metal catalyzed oxidation by mitochondria [9-10]. Fatty acids are separated from fat by the action of lipase and become free fatty acids, which have great biological activity potential [11].

The biological activity of free fatty acids plays a role in host defense against opportunistic microorganisms or pathogenic microorganisms in humans, and it is important to inhibit growth or destroy bacteria rapidly, as reported by [12], who found that gram-positive bacteria are more sensitive than gram-negative bacteria, and many fatty acids, such as palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and -linolenic acid, can act as surfactants (anions) and have antibacterial and antifungal properties. These elements may be factors that affect fatty acids. It has been shown that palmitic acid has antibacterial activity [13-14], then LA can be used as a cleaning agent and one of the natural products without chemical residues and this study aimed to detect the antibacterial activity of palmitic and lauric acid to inhibit *E. coli* and *S. aureus*.

Research Objectives

To evaluate the antibacterial activities and proper concentrations of commercial palmitic and lauric acids against *S. aureus* and *E. coli*.

To develop the food grade disinfectant of palmitic and lauric acid to reduction the concentration of *E. coli* and *S. aureus*.

Table 1: The concentration ratio of palmitic acid (A) combination with lauric acid (B) by using the experimental plan of 62 Factorial designs in Completely Randomized Design (CRD).

Factor	Independent variable	Level of factor (g/ml)					
Palmitic acid	A	0	0.2	0.4	0.6	0.8	1.0
Lauric acid	B	1.0	0.8	0.6	0.4	0.2	0.0

Factor	Ratio of fatty acid (g/ml)					
	A_1	A_2	A_3	A_4	A_5	A_6
B_1	0.0:1.0	0.2:1.0	0.4:1.0	0.6:1.0	0.8:1.0	1.0:1.0
B_2	0.0:0.8	0.2:0.8	0.4:0.8	0.6:0.8	0.8:0.8	1.0:0.8
B_3	0.0:0.6.8	0.2:0.6	0.4:0.6	0.6:0.6	0.8:0.6	1.0:0.6
B_4	0.0:0.4.8	0.2:0.4	0.4:0.4	0.6:0.4	0.8:0.4	1.0:0.4
B_5	0.0:0.2.8	0.2:0.2	0.4:0.2	0.6:0.2	0.8:0.2	1.0:0.2
B_6	$0.0:0.0^{Con*}$	0.2:0.0	0.4:0.0	0.6:0.0	0.8:0.0	1.0:0.0

A: Palmitic acid (mg/ml); An: Palmitic acid concentration (g/ml); B: Lauric acid; Bn: Lauric acid concentration (g/ml); n: run of sample; Con*: negative control is DMSO (non-combine of palmitic acid and lauric acid there is only DMSO for negative control).

Research Methodology

1) Materials

The palmitic acid and lauric acid from palm kernel oil were brought from the World Chemical Fareast Co., Ltd. They were prepared by dissolving one gram of fatty acid in one milliliter of dimethyl sulfoxide (DMSO) (RCI Labscan, Bangkok, Thailand) to a final concentration of one g/ml (w/v) (Table 1)

2) Determination of growth curve of *Escherichia coli* and *Staphylococcus aureus*

2.1) Microorganism Preparation

The two cultures of *E. coli* and *S. aureus* were kindly supported from Food Science and Technology laboratory, Division of Food Science and Technology, Agro-Industry, Chiang Mai University.

The streaking plate method was used to cultivate a single colony of purified *E. coli* and *S. aureus* and kept to stock cultures by using Mueller-Hinton broth (MHB) (R211443; Becton, Dickinson and Company Sparks, MD 21152 USA) and incubated for 18 h at 37 °C. Using the half-fold serial dilution method, the suspension was adjusted to a concentration that was a mix of MHB and 0.85 percent NaCl and then added to a 96 well microplate. Next, 20 µL of each microbial suspension was added to each well and incubated for 24 h at 37 °C. The turbidity of the suspension was adjusted using the optical density method using a microplate reader (EZ Read 2000, Biochrom, Holliston, MA, USA), and the UV-Vis absorbance was measured at 630 nm to obtain the initial cell at O.D. = 0.1 to achieve the 10^6 colony-forming units (CFU). To confirm the concentration of bacteria, the spread plate method was used to quantify the amount of *E. coli* and *S. aureus* by counting colony forming units (CFU) [15].

2.2) Preparation of the bacterial growth curve

Purified *E. coli* and *S. aureus* were grown in the MHB medium for 18 h at 37 °C. The concentration was adjusted by mixing MHB and 0.85 percent NaCl in a 96-well plate using the half-fold serial dilution method with the bacterial concentration set at 1 percent in each well. Then, the samples were incubated at 37 °C for 0, 2, 4, 6, 8, and 10 h. Bacterial samples were collected every two hours to measure the turbidity of the cells using the optical density method and the UV-Vis absorbance at 630 nm in accordance with the number of bacteria confirmed by the spread plate method at hours 6, 8, and 10 (modification method from [15].

3) Antibacterial Activity

3.1) Antimicrobial activity test of palmitic and lauric acid compare with commercial disinfectants by agar disc diffusion.

Swabbing *E. coli* or *S. aureus* on Mueller-Hinton agar (MHA) (CM0337, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK) from the starter culture (MHB and 0.85 percent NaCl), then waiting for 15 min and dropping 10 µL of PA combined with LA at various concentrations, as shown in Table 1 and DMSO with 10 µL for negative control on MHA, then putting all three substances Amoxycillin (0.03 mg/mL) (CT0223B, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK), Chloramphenicol (30 µg) (CT0013B, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK), and Tetracycline (30 µg) (CT0054B, Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK) of disc on the surface for positive control. After incubation at 37 °C for 20 h, the diameter of the inhibition zone was measured and the qualification ratio of fatty acids in the high inhibition zone was calculated, then agar disc diffusion

method it is a preliminary screening for antimicrobial activity which qualitatively the result whether the pathogen is susceptible to the test, and the experiment was repeated in three replications [16].

3.2) Minimum Inhibitory Concentration (MIC)

The purified *E. coli* and *S. aureus* strains were grown in MHB for 6 h at 37 °C, then adjusted using the McFarland standard no.5, the cultures turbidity was adjusted to 10^8 CFU/mL using sterile 0.85 percent NaCl (R20410, Lenexa, Kansas 66215, UK). Three-dimensional swabs were obtained over the entire surface of the MHA medium using a sterile cotton bud dipped in the suspension. Allow the surface to dry for 3-5 minutes. A sterile corker borer was used to press wells on agar with a diameter of 4 mm. Each of formulation of fatty acid concentration was added at 20 µL and diluted the concentration of each formulation by 0.5-fold dilution method. The MIC results were determined by examining the inhibition zone of the tested strain and measuring the diameter size of the tests was carried out in triplicate. The MIC of fatty acid was the lowest fatty acid content that did not allow culture to growth and the experiment was repeated in three replications [17].

3.3) Minimum Bactericidal Concentration (MBC)

The streak plate method was used to calculate the MIC results for MBC values by picking the colony from the MIC inhibition zone with the lowest concentration, at least three concentrations, streaking on the MHA, incubating at 37 °C for 24 h, and monitoring and recording bacterial growth. Fatty acids that destroy bacteria do not allow bacteria to thrive on MHA. The tests were carried out in triplicate, and the MBC of fatty acid was calculated using the lowest fatty acid content at which 99.9% or more of the initial inoculum was destroyed and the experiment was repeated in three replications [17].

4) Statistical Analysis

The experimental plan of Factorial designs in Completely Randomized Design (CRD) was designed for varying the concentration ratio of PA (A) combination with LA (B). All measurements were performed at least in triplicates. The means and standard deviations (± SD) were calculated. Significant differences were determined by analysis of variance (ANOVA) and Duncan's multiple range tests at 95% confidence intervals using SPSS software version 17.0. (IBM, U.S.A.)

Research Methodology

1) Growth curve of *E. coli* and *S. aureus*

The growth curves of *E. coli* and *S. aureus* were investigated. The growth curves of *E. coli* and *S. aureus* at 10 h show that the bacteria entered the stationary phase, as shown in Figure 1 and 2. Colonies of both bacteria were graphed at 10 h and the bacterial population was constant. This indicates that the bacteria on longer multiply, or the spawn rate, is equal to the mortality rate of the bacteria growing into the stationary phase because the waste produced by the bacteria inhibits bacterial growth. At 8 h, the bacteria were in the log phase with approximately 10^8 and 10^9 log CFU/ml, respectively, as shown in Figure 3 and 4.

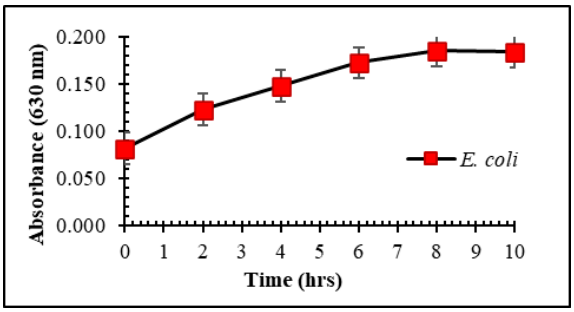

Figure 1: Growth curve of *E. coli* at 0, 2, 4, 6, 8 and 10 hrs.

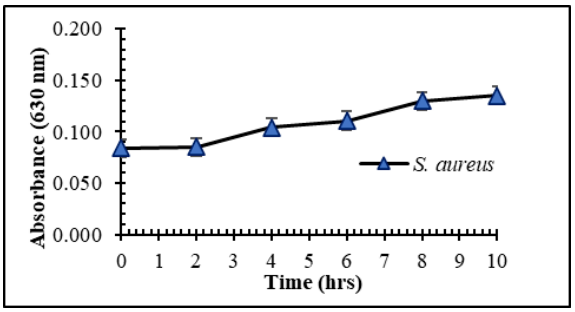

Figure 3: Growth curve of *S. aureus* at 0, 2, 4, 6, 8 and 10 hrs.

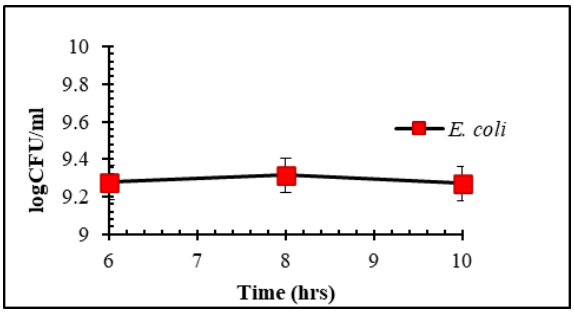

Figure 2: Number of *E. coli* colonies from spread plate technique at 6, 8 and 10 hrs.

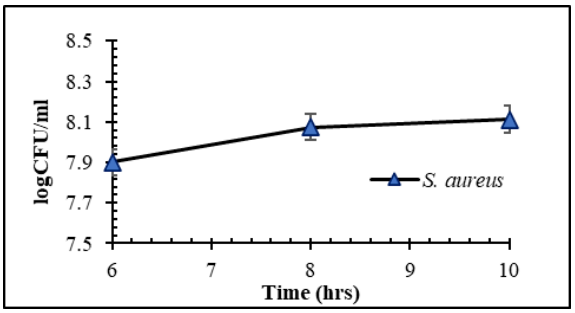

Figure 4: Number of *S. aureus* colonies from spread plate technique at 6, 8 and 10 hrs.

2) Antibacterial Activity

2.1) Antimicrobial activity test of palmitic and lauric acids compared with commercial disinfectants using the agar disc diffusion method

Table 2: Sensitivity analysis of commercial antibiotics.

Strains	Inhibition zone diameter (mm)		
	Amoxycillin (0.03 mg/mL)	Chloramphenicol (30 µg)	Tetracycline (30 µg)
E. coli	24.73 ± 0.35^{cB}	30.37 ± 0.90^{aA}	28.33 ± 1.29^{bA}
S. aureus	40.37 ± 1.06^{aA}	22.03 ± 0.31^{cB}	23.27 ± 0.60^{bB}

a, b, c ... in horizontal letters; then, A and B vertical letters show a statistically significant difference ($p < 0.05$) by Duncan's test from SPSS software version 17.0

Table 3: Sensitivity analysis of palmitic and lauric acids ratio.

Factor	Inhibition zone (mm)					
	E. coli					
	A_1	A_2	A_3	A_4	A_5	A_6
B_1	7.32 ± 0.20^{ghijk}	6.41 ± 0.20^{lm}	10.57 ± 0.40^{a}	8.38 ± 2.40^{ab}	6.64 ± 0.47^{klm}	6.72 ± 0.47^{ijklm}
B_2	10.40 ± 0.57^{a}	10.59 ± 1.08^{a}	8.64 ± 0.39^{b}	8.89 ± 0.38^{b}	8.46 ± 0.35^{bc}	7.89 ± 0.84^{cde}
B_3	8.71 ± 0.40^{b}	7.69 ± 0.27^{def}	7.14 ± 0.52^{ghijk}	7.80 ± 0.48^{cdef}	7.36 ± 0.49^{ghij}	7.31 ± 0.46^{ghijk}
B_4	8.28 ± 0.25^{bcd}	7.41 ± 0.28^{ghi}	7.42 ± 0.47^{ghi}	7.37 ± 0.46^{ghij}	7.43 ± 0.59^{ghi}	7.63 ± 0.85^{defg}
B_5	8.26 ± 0.58^{bcd}	6.97 ± 0.51^{ghijkl}	6.68 ± 0.58^{jklm}	6.33 ± 0.36^{lm}	6.39 ± 0.39^{lm}	6.40 ± 0.32^{lm}
B_6	0.00^{Con}	6.83 ± 0.19^{hijkl}	7.29 ± 0.57^{hijk}	6.88 ± 0.32^{hijkl}	6.96 ± 0.55^{ghijkl}	6.11 ± 0.03^{m}
Factor	Inhibition zone (mm)					
	S. aureus					
	A_1	A_2	A_3	A_4	A_5	A_6
B_1	13.92 ± 1.51^{a}	11.73 ± 0.51^{cde}	10.98 ± 0.59^{fgh}	6.26 ± 0.09^{n}	6.62 ± 0.22^{mn}	11.37 ± 0.68^{de}
B_2	8.67 ± 0.98^{k}	6.38 ± 0.29^{n}	6.92 ± 0.73^{mn}	6.67 ± 0.36^{mn}	7.60 ± 1.06^{l}	10.91 ± 0.66^{fgh}
B_3	13.54 ± 0.65^{ab}	11.63 ± 0.46^{cdef}	11.30 ± 0.75^{defg}	11.44 ± 1.04^{def}	12.17 ± 0.06^{c}	11.21 ± 0.85^{efg}
B_4	12.97 ± 0.77^{b}	11.97 ± 0.54^{cd}	11.44 ± 0.44^{def}	10.42 ± 0.37^{hi}	10.88 ± 0.76^{fgh}	9.40 ± 0.78^{j}
B_5	10.34 ± 0.64^{hi}	10.61 ± 0.67^{gh}	10.98 ± 0.97^{fgh}	9.91 ± 0.80^{ij}	9.49 ± 0.87^{j}	9.22 ± 0.55^{jk}
B_6	0.00^{Con}	6.32 ± 0.18^{n}	6.48 ± 0.29^{mn}	7.51 ± 0.71^{l}	7.13 ± 0.61^{lm}	6.37 ± 0.30^{n}

a, b, c ... in letters show a statistically significant difference ($p < 0.05$) by Duncan's test from SPSS software version 17.0; An, palmitic acid concentration (g/ml); Bn, lauric acid concentration (g/ml); n: run of sample; Con*, negative control is DMSO.

The effects of PA and LA were tested at different ratios using the disc-diffusion method. It

Table 4: MIC and MBC values of palmitic acid in combination with lauric acid.

Formula	*E. coli*		*S. aureus*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
A_1B_2	200	800	50	200
A_2B_2	62.5	125	62.5	250
A_3B_1	87.5	175	87.5	175

negative control is DMSO; AnBn: combination of palmitic and lauric acid ratios; n: run of sample.

inhibited the growth of *E. coli* and *S. aureus*, as shown in Table 3. In addition, when comparing the inhibitory activity between PA and LA with commercial antibiotics including Amoxycillin (0.03 mg/mL), Chloramphenicol (30 µg) and Tetracycline (30 µg) for inhibition of *E. coli* and *S. aureus* (Table 2).

The optimum ratio for inhibiting *E. coli* was A3B1, A1B2, and A2B2; the PA to LA ratios were 0.4:1.0, 0:0.8, and 0.2:0.8 g/ml and the optimal ratio for inhibition of *S. aureus* was A1B1 and A1B3; and the ratio between PA and LA was 0:1.0 and 0:0.6 g/ml as shown in Table 3. This is the most effective ratio of the inhibition zone for *E. coli* and *S. aureus* for studying the effects of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) because from the result finding for optimum growth ratio due to *E. coli* to be stronger and can increase resistant to antibiotic, than *S. aureus*. Therefore, a disinfectant formula that has a high in inhibition zone with *E. coli* is used to select the highest optimum ratio for inhibition bacteria.

2.2) Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of palmitic acid combined with lauric acid

The study results in effect of MIC and MBC on inhibition of *E. coli* and *S. aureus* were showed in Table 4, it was found that the optimal ratio of PA and LA to inhibit and destroy both of *E. coli* and *S. aureus* was A3B1 due to the highest inhibition ability affect to *E. coli* and *S. aureus* compared to A1B2 and A2B2, and giving the diameter of MIC of A3B1 , A1B2, and A2B2 with concentration raged between 50-800 mg/ml at 8.85±0.43, 9.30±0.67, 8.42±0.67, 9.58±0.49, 8.13±0.25 mm., and 11.37±0.56, 13.15±0.68, 13.22±0.45, 14.25±1.26, 14.09±0.91 mm., respectively, as shown in Table 5.

Table 5: Diameter of MIC of palmitic combination with lauric acid.

Diameter of MIC (mm)					
E. coli					
A_1B_2		A_2B_2		A_3B_1	
Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC
800	12.22 ± 0.98^{a}	1000	10.83 ± 1.61^{a}	1400	8.85 ± 0.43^{ab}
400	10.42 ± 0.65^{b}	500	9.07 ± 0.28^{b}	700	9.30 ± 0.67^{a}
200	8.72 ± 1.50^{c}	250	9.00 ± 0.45^{b}	350	8.42 ± 0.67^{bc}
100	NA	125	8.33 ± 0.69^{c}	175	9.58 ± 0.49^{a}
50	NA	62.5	6.23 ± 0.09^{d}	87.5	8.13 ± 0.25^{c}
25	NA	31.25	NA	43.75	NA
S. aureus					
A_1B_2		A_2B_2		A_3B_1	
Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC	Concentration (mg/ml)	MIC
800	14.85 ± 0.46^{ab}	1000	10.23 ± 0.40^{c}	1400	11.37 ± 0.56^{c}
400	11.50 ± 0.87^{c}	500	13.10 ± 0.62^{b}	700	13.15 ± 0.68^{b}
200	15.33 ± 0.49^{a}	250	12.97 ± 1.09^{bc}	350	13.22 ± 0.45^{b}
100	12.35 ± 0.32^{b}	125	14.18 ± 0.34^{a}	175	14.25 ± 1.26^{a}
50	10.32 ± 1.32^{d}	62.5	13.92 ± 1.85^{ab}	87.5	14.09 ± 0.91^{a}
25	NA	31.25	NA	43.75	NA

Values with different superscripts between rows differ significantly at $P<0.05$ by Duncan's test from SPSS software version 17.0; NA: no activity; negative control: DMSO; AnBn: combination of palmitic and lauric acid ratios; n: run of sample.

Discussion

1) Growth curve of *E. coli* and *S. aureus*

From figure 1-4, where the log-phase is when bacteria multiply the most, then have a constant rate of division of chemical components of cells and processes as well as the same physiological

properties. Bacteria cells intensely increased in size, specially the first hour and from 3 to 6 h, have cells size increasing and at 10 h can see of dead cells and the growth curve of *E. coli* at a constant temperature [18,28], it was observed that there was an increase in growth over an increased period of time, and at 6 hours *E. coli* ranged between 10^6 to 10^7 logCFU [19], which is consistent with the results of our experiments. The growth curves of *E. coli* and *S. aureus* measured by UV-Vis absorbance at 620 nm showed that both bacteria grew similarly. It is an exponential increasing (log phase) during, and the first 6 to 8 hours are stable stationary phase, log phase for *E. coli* appears to be longer than that measured for *S. aureus* [15], but log phase of *E. coli* this research are shorter than *S. aureus* because the condition experimental are difference. Therefore, the optimal time of inoculation to test the efficacy of the antimicrobial palmitic acid and lauric acid was between 8 h, since the bacteria were in the log phase. In this result is conducted an experiment of growth curve at 10 h because both bacteria are growth to log-phase prior to the stationary phase at 8 h.

The log-phase of growth curve higher to stationary phase and the log-phase prior to the stationary phase the bacteria are higher in activity and resistance to conditions than the other phases due to due to the number of bacteria at stationary phase are relatively constant and the number of bacteria that grown is same as the number of bacteria that die, which was effect from lack of nutrients or accretion of toxic metabolic products [20,30], then were chosen at log-phase prior to the stationary phase for the three formula of food grade disinfectant tests in antimicrobial.

2) Antibacterial Activity

PA and LA were found to be highly inhibited compared to commercial antibiotics, and from the result found that *E. coli* can be resistant to antibiotic and disinfectant than *S. aureus* and our result agrees with findings of similar studies by Bantawa, K. et al., (2019), Acsa, I. et al., (2021) and Sheikh, A. A. et al., (2012) because there is a difference in mode of reaction of Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) to disinfectants, then affect to *E coli* were more resistant to the disinfectants compared to *S. aureus* and Gram-positive bacteria tend to be more responsive to disinfectant compared to Gram-negative bacteria due to intrinsic factors and difference in cell structure between Gram-positives and Gram-negatives, and found that the cell wall of Gram-positive bacteria does not action as barrier for disinfectants which difference to Gram negative [21-22,29]. According to other research found that PA and LA are Saturated Fatty Acids (SFAs) that can act as antimicrobial agents (as shown in Table 2-3) [23-24]. Both types of bacteria are sensitive to fatty acids due to Gram-negative and Gram-positive bacteria are inhibited differently by different types of fatty acids due to the permeability of the outer membrane of Gram-negative bacteria, which acts as a barrier for Gram-negative bacteria [24]. When comparing the antibacterial effects of PA and LA, and then

LA inhibited *E. coli* and *S. aureus* better than PA, and the combination of PA and LA promoted the inhibitory effect of both types of bacteria. The results in table 3 found that the most appropriate ratio of PA and LA to inhibit *S. aureus* and *E. coli* was 0:1.0 and 0:0.6 g/ml, respectively. The reactive oxygen species (ROS) from fatty acid synthase (FAS) complex of PA and LA increases cellular toxicity and the rate of cell damage due to genes responsible for fatty acid desaturation; damage to DNA, RNA, and proteins [8-10], and then effect to Inhibit gram-negative and gram-positive bacteria. The phenomenon can be explained caused by Palm Kernel Oil (PKO) was active against certain strains of microorganisms, including *E. coli* and *S. aureus* [26], and Palm Oil (PO), Red Palm Oil (RPO), and PKO have antibacterial activity against gram-positive bacteria such as S. aureus because they contains a variety of fatty acids that can inhibit bacteria, including PA and LA contained in PO, RPO, and PKO [5]. When tested for the antibacterial activity of PKO using the disc diffusion method, moderately sensitive and highly sensitive antibacterial activity of *E. coli* and *S. aureus*, respectively, showed that PKO was more effective in inhibiting the bacteria [2] and LA affect to growth of *S. aureus*, and *E. coli* which LA at 5%, 10%, 15%, and 20% dissolved in n-hexane as a solvent based on the disc diffusion method was determined by measuring the diameter inhibition zone to inhibit the growth of bacteria such as S. aureus with diameters of 40, 37, 28, and 25 mm, respectively, and *E. coli* with diameters of 41, 36, 28, and 26 mm, respectively, showing that LA had the best inhibitory effect on *E. coli* and *S. aureus* [7] and consistent this research.

And the MIC of bacteria was 87.5 mg/ml (Table 4) due to the high LA concentration ratio and the ability to inhibit bacteria better than PA. From the Table 5, It was found that the ratio of A3B1 has the best MBC were 87.5 and 175 mg/ml, then compared the results MIC test of other research found that PO, RPO and PKO with PA constituents were 42.93%, 41.96% and 9.46%, respectively, and the composition of LA was 0.20%, 0.23% and 45.24%, respectively, the effect of MIC from PO, RPO and PKO are less than 4.50, 4.50 and 1.13 mm, respectively [5], It has less antibacterial effect than using PA and LA directly, then from study result concluded that *E. coli* was inhibited by the PKO because of contains a main fatty acids such as PA and LA and the inhibition zone was 5 mm [5,27]. Therefore, PA and LA from this study were used to inhibit and destroy *E. coli,* and *S. aureus* was found to be more efficient than PO, RPO, or PKO.

Conclusion

In conclusion, palmitic acid (PA) and lauric acid (LA) were more effective than palm oil (PO), refined palm oil (RPO), and palm kernel oil (PKO) at inhibiting and disinfecting *E. coli* and *S. aureus*. The

efficacy of lauric acid enhances that of palmitic acid, thereby affecting the inhibition and disinfection effects on bacteria. The combination of these fatty acids proved more effective than using palmitic acid alone. Therefore, with the increasing demand to reduce the use of chemical cleaning agents in the food industry, it is necessary to develop food-grade disinfectants capable of inhibiting *E. coli* and *S. aureus*.

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