

Antibacterial activity of Enzymatic hydrolyzed of Virgin Coconut oil and Palm Kernel oil against *Staphylococcus aureus*, *Salmonella thypi* and *Escherichia coli*

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Abstract: Triglycerides and diglycerides do not show antibacterial activity, but free fatty acids and monoglycerides, especially lauric acid and monolaurin do. The purpose of this study was to determine the antibacterial activity of the enzymatic hydrolyzed of virgin coconut oil (VCO) and palm kernel oil (PKO), which produce a combination of lauric acid and monolaurin. Hydrolysis process was done by the enzyme lipase (Lipozym TL IM) which was active at 1.3 position. The hydrolyzed oil then tested for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella thypi* using agar diffusion method with paper disc with diameter of 6 mm (Oxoid, England). Antibacterial activity test carried out on VCO and PKO, hydrolyzed VCO (VCOH) and PKO (PKOH) each at 25% concentration, 50%, 75% and 100%. Antibacterial activity of the test material compared with chloramphenicol (30 µg) and tetracycline (30 µg). The results showed that the optimum hydrolysis time is 14 hours. There is low and similar antibacterial activity of VCO and PKO. VCOH and PKOH show higher antibacterial activity than VCO and PKO. There is an increase of antibacterial activity by increased levels of VCOH and PKOH. Antibacterial activity against *S. aureus* (Gram positive) was higher than *S. thypi* and *E. coli* (Gram negative). Antibacterial activity of the test material is lower than the standard chloramphenicol and tetracycline .

Keyword: antibacterial, lipozyme, palm kernel oil, partial hydrolysis, virgin coconut oil.

INTRODUCTION

Virgin Coconut Oil (VCO) is obtained from the flesh of fresh coconuts (*Cocos nucifera*) processed at low temperature or without heating and Palm Kernel Oil (PKO) obtained from palm kernel (*Elaeis guineensis*). Both oil are called lauric oils because the similarity of medium chain fatty acid composition, lauric acid, reaches 50%.¹ Lauric acid, which containing 12 carbon atoms, belongs to medium chain fatty acids (MCFA). These fatty acids are bound in the form of triglycerides. In the human body triglyceride is converted into monoglycerides and fatty acids, which is monolaurin and lauric acid, that have antiviral, antibacterial, and antiprotozoa activity.²

Antibacterial activity of PKO was tested before and the results showed that PKO have antibacterial activity against *Escherichia coli* while palm oil do not.³ Other studies have also shown the ability of PKO inhibition against *Staphylococcus aureus* and *Streptococcus sp.*² VCO can inhibit the growth of pathogenic bacteria such as *Listeria monocytogenes*, *Staphylococcus sp* and *Helicobacter sp.*^{3,4,5}

Fat is converted into fatty acids and monoglyceride by saponification or by enzymatic process.⁶ The partial hydrolysis of VCO can increase the inhibition of bacterial, either hydrolysis by the enzyme lipase (lipozyme) or with NaOH (saponification). The best result was shown by enzymatic hydrolysis method with incubation time of 12 hours. The increased enzymatic incubation time is proportional to the increase in free fatty acid content in VCO and an increase in antibacterial activity.⁷

If consumed, fat is converted into fatty acids and monoglyceride by the enzyme lipase in the digestive track. There are three sources of lipase enzymes that hydrolyze fat before it is absorbed in the intestinal digestion, the lingual lipase, gastric lipase and pancreatic lipase. Lipase can hydrolyze fatty acid partially, for short, medium and long fatty acid, which is at sn-1,3 position. Thus if VCO and PKO consumed, they will undergo partial hydrolysis by the enzyme lipase in the digestive tract before it is absorbed.⁸

The purpose of this study was to determine the antibacterial activity of enzymatic hydrolyzed of VCO and PKO performed using the enzyme lipase (Lipozyme TL IM) against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella thypi*.

EXPERIMENTAL

Apparatus

The tools used in this research were included shaker (Combi-shaker, Germany), incubator (Sonica, Japan), maximum power balance 220 g (Precisa, Japan), water bath, burette, statip, clamps, autoclave, turbo mixer, incubators, glassware and tools as needed in research.

Materials

The chemicals used in this study, unless otherwise stated, are pro-analysis quality from E. Merck (Germany), including n-hexane, tris-hidroksimetilaminometan, sodium hydroxide, ethanol, hydrochloric acid, calcium chloride, sodium sulfate anhydrous, phenolphthalein indicator (1% in alcohol), sodium chloride and Lipozyme TL IM. Materials for antibacterial activity were included nutrient agar and mueller hinton agar (Oxoid, England) and bacteria used were *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 10536) and *Salmonella thypi* (ATCC 786). Mc Farland Equivalence Turbidity Standard (Remel, USA). Paper disc were 6 mm diameter (Oxoid, England). Reference standard tetracycline and chloramphenicol (Oxoid, England). VCO is produced by PT. Siti Nurbaya, West Sumatra, and PKO used is from PT. Wilmar, Medan, North Sumatera.

Method

There were two stages in this study, the first was process of enzymatic hydrolysis of the VCO and PKO, and the second was antibacterial activity of hydrolyzed oil.

Hydrolysis process by the enzyme lipase (Lipozym TL IM), which were active at 1.3 position conducted for 18 hours. A total of 30 grams of oil, to which added 30 grams of distilled water, 12.5 ml of 0.063M CaCl₂, 25 ml of Tris-HCl buffer, 500 miligrams of Lipozyme TL IM, in an erlenmayer flask. The material shaken 10 minutes at 200 rpm. Subsequently incubated at 55°C, and was shaken every hour for 10 minutes at 200 rpm.^{9,10} An increase of the acid number of free fatty acid determined every 2 hours by titration.

Hydrolyzed oil then tested for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella thypi* using agar diffusion method with 6 mmdiameter paper disc (Oxoid, England).¹¹ Antibacterial activity test carried out on unhydrolyzed VCO and PKO, hydrolyzed oil of VCO (VCOH) and PKO (PKOH) each with concentration of 25%, 50%, 75% and 100%. Antibacterial activity of the test material compared with chloramphenicol (30 µg) and tetracycline (30 µg).

The data than analyzed using Analysis of Variance (ANOVA).¹²

RESULT AND DISCUSSION

Enzymatic Hydrolysis

Hydrolysis enzymatic process carried out during the 18-hour incubation period with the observation time interval every 2 hours to determine the optimal incubation time of the enzymatic reaction between Lipozyme with each oil, VCO and PKO. The optimal incubation period is determined when a constant acid number reached which indicates that no longer significantly increase amount of free fatty acids. The result presented in Table 3.1 and Figure 3.1.

Table 3.1 Acid Numbers and incubation time of hydrolysis of PKO and VCO

Method	Incubation time	Mean ± SD Acid number (mg NaOH/g Oil) n = 3	
		VCO	PKO
Non hydrolysis oil	-	0.33 ± 0.34	0.24 ± 0.28
Enzymatic hydrolysis oil	0 hour	4.78 ± 0.69	4.33 ± 0.70
	2 hours	10.82 ± 2.15	10.32 ± 2.84
	4 hours	30.03 ± 2.16	21.74 ± 1.38
	6 hours	48.25 ± 2.69	35.75 ± 2.69
	8 hours	89.97 ± 1.48	78.43 ± 3.05
	10 hours	115.53 ± 0.97	108.68 ± 1.92
	12 hours	131.67 ± 1.41	125.05 ± 1.34
	14 hours	141.96 ± 1.27	136.09 ± 1.14
	16 hours	142.58 ± 1.24	137.15 ± 0.89
	18 hours	143.58 ± 1.52	137.37 ± 0.66

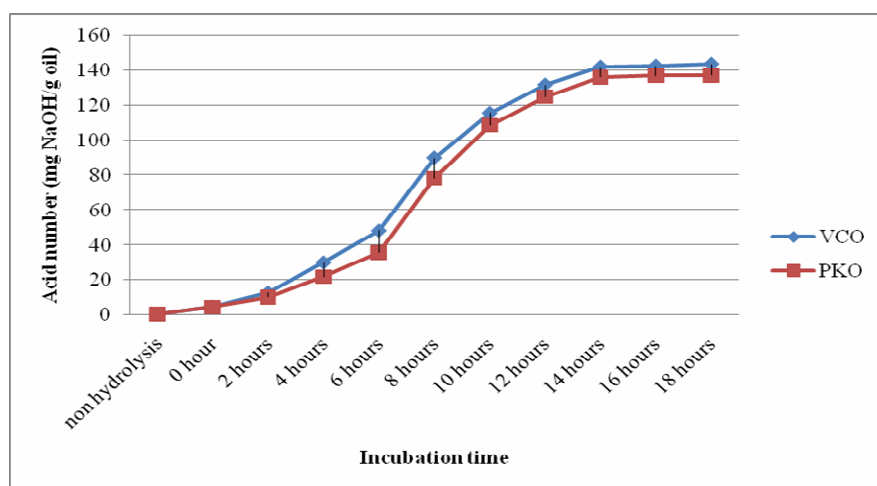


Figure 3.1 Chart of Acid Number and incubation time of hydrolysis of VCO and PKO

Acid number is amount of free fatty acids contained in the oil or fat. Enzymatic hydrolysis VCO and PKO with Lipozyme TL IM will produce 2 free fatty acids molecules and 1 monoglyceride from each triglyceride molecule present in the VCO and PKO, because Lipozyme works specifically on position 1 and 3 of triglycerides.⁶ Lipozyme TL IM works like the lipase enzymes in the human gastrointestinal tract.

The increasing of acid number can be seen from Table 3.1 and Figure 3.1, it showed that there were increasing acid number during the 14 hours. After 14 hours there is no significant increase in acid number. This suggests that the optimum condition of enzymatic reactions occurred significantly in 14 hours and the end point is reached when the enzymatic reaction incubation period of 14 hours.

The main content of lauric oil is lauric acid, so the results of hydrolyzed VCO and PKO with lipase enzyme which is active at sn-1.3 position is mainly lauric acid and monolaurin, which acts as an antibacterial.^{13,14,15,16}

Antibacterial Activity

The antibacterial activity test is determined by the inhibition zone that formed around the paper discs, and the result can be seen in Figure 3.2, Table 3.2 and Figure 3.3.

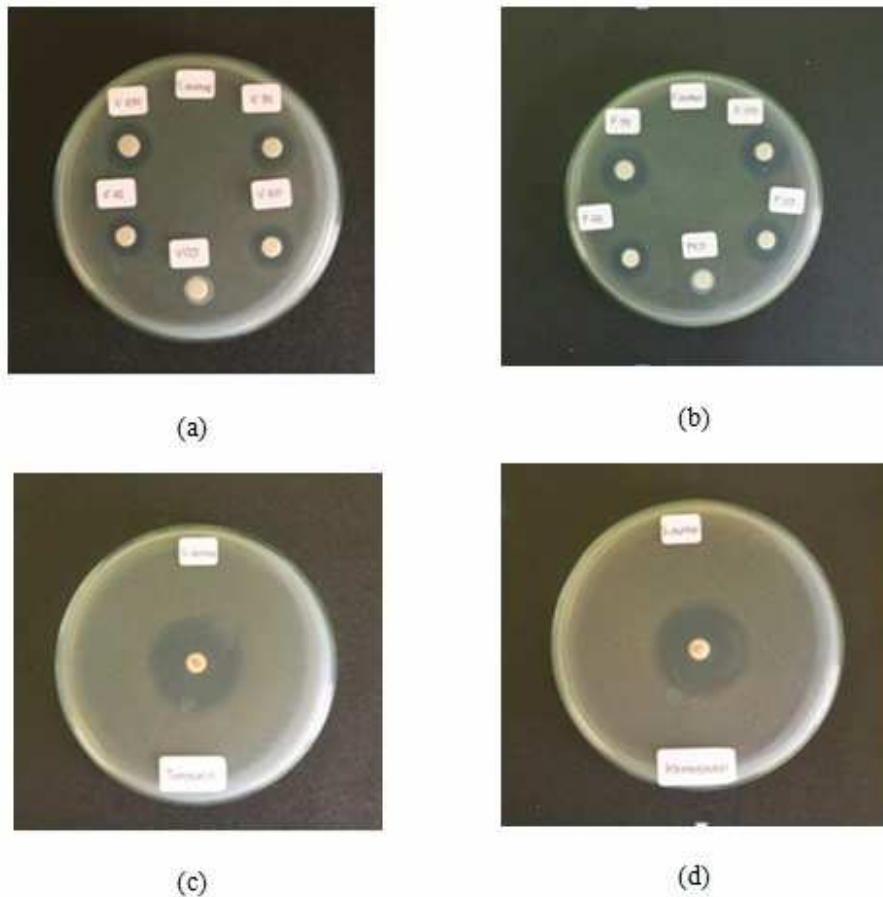


Figure 3.2 Inhibitory Zone of VCO (a), PKO (b), tetracycline (c) and chloramphenicol (d) against *S. aureus*

Table 3.2 Antibacterial activity of the test substance against *S. aureus*, *S. thypi*, and *E. coli*

Sampel	Level (il/ml)	Mean ± SD Inhibition Zone (mm) n=3					
		<i>S. aureus</i>		<i>S. thypi</i>		<i>E. coli</i>	
		VCO	PKO	VCO	PKO	VCO	PKO
Non hydrolysis oil	100%	10.02 ± 0.48	9.6 ± 0.17	9.07±0.15	8.43 ± 1.15	8.17 ± 0.65	7.83 ± 0.85
Enzymatic hydrolysis oil	25%	13.57 ± 0.35	12.75 ± 0.13	10.53 ± 0.91	9.20 ± 1.22	9.13 ± 0.40	9.07 ± 0.67
	50%	14.57 ± 0.15	13.73 ± 0.57	11.80 ± 1.15	10.10 ± 0.87	10.05 ± 0.87	10.47 ± 0.38
	75%	15.74 ± 0.39	15.02 ± 0.97	13.00 ± 0.79	11.33 ± 0.97	11.93 ± 0.51	11.4 ± 0.26
	100%	17.17 ± 0.13	16.32 ± 0.45	13.77 ± 0.31	12.83 ± 0.12	13.13 ± 0.32	12.63 ± 0.21
Tetracycline	30 ìg	26.42±0.16		27.27±0.38		29.43±0.46	
Chloramphenicol	30 ìg	30.40±0.43		34.233±0.30		32.15±0.25	

Note: diameter of paper disc is 6mm

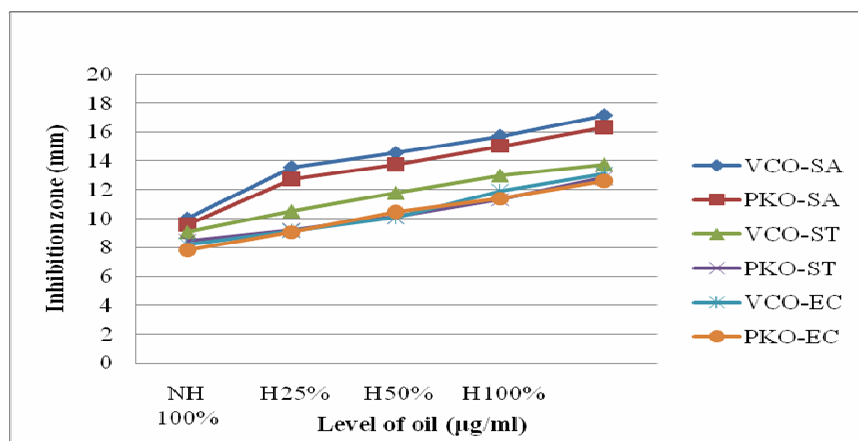


Figure 3.3 Chart of levels of hydrolysis oil and inhibitory zone of VCO against *S. aureus* (VCO-SA), *S. thypi* (VCO-ST), *E. coli* (VCO-EC) and PKO against *S. aureus* (PKO-SA), *S. thypi* (PKO-ST), *E. coli* (PKO-EC). NH = non hydrolysis oil, H = Hydrolysis oil

The result of antibacterial activity test shows that VCO and PKO give the minimal and similar antibacterial activity against *S. aureus*, *E. coli* and *S. thypi*. This is consistent with previous research that says that PKO provide inhibitory zone against *E. coli*.¹⁷ Antibacterial activity against *S. aureus* (gram-positive) is higher than against the *S. thypi* and *E. coli* (gram-negative). As seen on Figure 3.3 the inhibition zone for *S. aureus* is higher.

There were significantly increased on antibacterial activity of its hydrolyzed oils, VCOH and PKOH. Elevated concentration of VCOH and PKOH provide significantly increased antibacterial activity. But there was no significant difference in antibacterial activity between VCOH and PKOH.

Of all saturated fatty acids, lauric acid has higher antimicrobial activity than caprylic acid (C8 : 0), capric acid (C10 : 0), and myristic acid (C14 : 0). In general, reported that the fatty acids and monoglycerides inactivates bacteria by damaging the plasma membrane (lipid bilayer).^{3,18,19}

The result of hydrolyzed oil are lauric acid and monolaurin. Monolaurin is a non-ionic surfactant having two ends with different properties, one end is hydrophobic and the other is hydrophilic.¹⁹ So that it can interfere the growth of bacteria, both gram- positive and gram-negative bacteria. Gram-negative bacteria outer membrane is lipopolysaccharide composed of lipids, polysaccharides and proteins, is more non - polar whereas gram-positive bacteria consists of a thicker peptidoglycan layer has a polar in nature, they can be affected by monolaurin. Gram-negative bacteria have thicker lipopolisacaride layers so that lauric acid and monolaurin easily penetrate through the membrane, and its equally non polar making pervasive, damaging the cell walls of bacteria, and bacteria die. As against gram-positive, although the membrane lining layer contains very little fat, but as monolaurin is surfactant so it can damage the bacterial cell membrane, resulted membrane lysis and then inhibited bacterial growth.

The benefit of these oils, VCO and PKO are food, not an antibiotic, so that intestinal bacteria tend to be more receptive.

CONCLUSION

The results showed that the enzymatic hydrolysis process VCO and PKO by Lipozyme TL IM is optimum for 14 hours. There is low and similar antibacterial activity of VCO and PKO. There is significant increase of the antibacterial activity by increased levels of VCOH and PKOH. Antibacterial activity against *S. aureus* (Gram positive) was higher than the *S. thypi* and *E. coli* (Gram negative), but the antibacterial activity of the test material is lower than tetracycline and chloramphenicol.

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