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Antibacterial Activity of Hydrolyzed Oils of Different Fatty Acid Composition against Salmonella Thypi and Lactobacillus Plantarum

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Abstract: Ttriglyceride and diglyceride do not have antibacterial activity, but free fatty acid and monoglyceride are antibacterial. The mixture of free fatty acid and their monoglycerides can be obtained by hydrolysis of the oil using specific enzyme. The aim of this study was to determine antibacterial activity of hydrolyzed oil with different fatty acid composition including medium chain triglycerides (MCT), and long chain triglyceride (LCT) against bacteria *Salmonella thypi* and *Lactobacillus plantarum.*

The materials used in this study were Virgin Coconut Oil (VCO), soybean oil, and palm oil. The oil was hydrolyzed using lypozyme which is active on *sn*-1 and *sn*-3 position. Hydrolyzed oil was extracted with n- hexane, evaporated, and then acid number was determined. Antibacterial activity of hydrolyzed oil was determined by diffusion agar method against *Salmonella thypi* (ATCC 19943) and *Lactobacillus plantarum* (ATCC 14917) at concentration of 25%, 50%, 75%, and 100%.

The results of the study show that antibacterial activity of hydrolyzed oils against tested bacteria is affected by the type and concentration of hydrolyzed oil. Hydrolyzed oil has antibacterial activity against tested bacteria. Antibacterial activity of hydrolyzed oil is more active against *Salmonella thypi* than *Lactobacillus plantarum*. The antibacterial activity against tested bacteria is shown to be highest by hydrolyzed VCO and followed by hydrolyzed soybean oil, and the lowest by hydrolyzed palm oil.

Antibacterial activity of hydrolyzed oil is influenced by the presence of double bond and the length of fatty acid chain in the oil; medium chain fatty acid has the highest activity, followed by unsaturated long chain fatty acid and the lowest by saturated long chain fatty acid.

Keyword: vegetable oil, lypozyme, hydrolysis, antibacterial, Salmonella, Lactobacillus.

Introduction

Antibacterial activity of fatty acids and, their derivatives such as monoglycerol esters (monoglyceride) and alcohol esters were studied in 1970s. It was reported that antibacterial activity of fatty acids were influenced by chain length and unsaturation. Of the saturated fatty acids, lauric acid (C12:0) was the most active against gram-positive microorganism. Monoenoic acid (C18:1) was more active than long saturated acid (C16:0), but less active than dienoic acid (C18:2). Of the monoglycerol esters, monoglycerol laurate (monolaurin) was the most active. Diglycerides and triglycerides were not active.[1,2].

Combination or mixture of free fatty acids and their monoglyceride can be obtained by two means, namely enzymatic method and partial hydrolysis with NaOH. Enzymatic hydrolysis with enzyme which specifically active to hydrolyze fatty acid at sn- 1 and sn- 3 position, and therefore does not hydrolyze fatty acid at sn- 2 position, so that will generate 2- monoacylglycerol (2- MAG) and two free fatty acids from one

triglyceride molecule. It was found that antibacterial activity of enzymatic hydrolysis of VCO with incubation time of 12 hours was more active than that by partial hydrolysis with NaOH through partial saponification.[3]

Antibacterial activity of hydrolyzed VCO was more effective toward pathogenic bacteria (*Salmonella*) than probiotic (*Lactobacillus*). [4,5]

The aim of this study was to determine antibacterial activity of enzymatic hydrolyzed vegetable oils with different fatty acid composition that is medium chain triglyceride VCO composed of medium chain fatty acid, long chain triglycerides including palm oil composed of long chain saturated fatty acids, and soybean oil composed of long chain unsaturated fatty acids against pathogenic bacteria *Salmonella thypi* and probiotic bacteria *Lactobacillus.* Antibacterial activity of tested hydrolyzed oils was preformed by diffusion agar method

Materials and Methods

Apparatus

Apparatus used in this study were analytical balance, laminar air flow cabinet, spectrophotometer, hotplate, autoclave, oven, incubator, water bath, and laboratory glassware.

Materials

Oils used in this study were palm oil (Sania), VCO (Palem Mustika) and soybean oil (Mama Suka). All chemical materials used in this study were pro- analysis quality from E.Merck (Germany) including calcium chloride, tris-hydroxymethylaminomethan, *Lipozyme*® TL IM, n- hexane, hydrochloric acid, anhydrous sodium sulfate, ethanol 96%, potassium hydroxide, phenolphalein (1% in alcohol), DMSO. Materials used to examine antibacterial activity were nutrient agar powder, lactose broth powder and, nutrient broth powder. Paper disc with diameter of 6 mm was used (OXOID). Bacteria used were *Salmonella typhi* ATCC 19943 and *Lactobacillus plantarum* ATCC 14917.

Hydrolysis of Oil

Fifty (50) gram of oil was placed in an erlenmeyer of 250 ml, to which then 50 ml of water, 12.5 ml $CaCl_2$ of 0.063 M, 25 ml buffer tris-HCl solution with pH of 8, and 500 mg lipozyme were added. The mixture was stirred to homogenous, then incubated at 40± 0.5^0C for 14 hours, and was stirred for 10 minutes in every 1 hour during incubation. The result was transferred in to separating funnel, extracted with 50 ml n- hexane which resulted in two layers. Upper layer (n- hexane fraction) was separated (filtrate 1). Bottom layer was extracted again with 50 ml n- hexane and separated again to obtain filtrate -2. Both filtrates were combined and to which anhydrous Na_2SO_4 added and allowed to stand for 15 minutes. It was evaporated on water bath to dryness to obtain hydrolyzed oil. [3] Acid value determination was done by transferring 10 gram hydrolyzed oil in to an erlenmeyer of 200 ml. Added 50 ml neutral alcohol of 95%, then warmed for 10 minutes on water bath while stirred. Then solution obtained was titrated with KOH of 0.1 N using phenolftalein of 1% in alcohol as indicator. The end point of titration was when the pink color appeared, then the acid value was calculated. [6]

$$\text{Acid value} = \frac{\text{A x N x 56.1}}{\text{G}}$$

Note:

A : Total volume of ml KOH used for titration

N : Normality of KOH solution

G : weight of hydrolyzed oil (gram)

Inoculum Preparation

A colony of *Salmonella thypi* bacteria was taken by sterile loop, then planted on the slant agar media by scratching, then incubated in incubator at 32- 35^0C for 24 hours. Inoculum was prepared by transferring bacteria from culture media, then planted in nutrient broth media, then incubated for 3-5 hours, until the transmittance was 25% (total bacteria of 10^6 cfu/ ml) measured at wave length of 580 nm. [3,7] Lactobacillus plantarum was prepared in the same manner using lactose broth instead of nutrient agar.

Antibacterial Activity Test

A volume of 0.1 ml inoculum was placed in sterile petri dish, to which 20 ml nutrient agar was poured at 40- 50^{o}C, then the petri dish was agitated on the table so the media and bacterial suspension mixed well. Paper disc was soaked in the tested material for ± 15 minutes. Soaked paper disc was placed on solidified media, then allowed to stand for ± 15, then incubated at 35±2^{0}C for 18- 24 hours. Diameter of clear area around each disc was measured as inhibition zone. Statistical analysis of data obtained was done using Tukey HSD. [8,9]

Results and Discussion

Enzymatic Hydrolysis of Oils

In this study, enzymatic hydrolysis was done on VCO, soybean oil, and palm oil. Acid values of the hydrolyzed oils were 148.1 mg KOH/g, 146.9 mg KOH/g and, 147.6 mg KOH/g for VCO, Soybean oil and Palm oil respectively. Acid value is a measure for total of free fatty acid contained in oil or fat which is defined as the number of milligrams of potassium hydroxyde required to neutralize the free fatty acids in 1 g of the sample. [6] Acid value of hydrolyzed oil was determined after complete hydrolysis by lipase enzyme for 14 hours as it was previously done by the others indicated by constant acid value after 14 hours. [8,10]

Antibacterial Activity of Hydrolyzed Oils

Antibacterial activity of hydrolyzed oil against pathogenic bacteria Salmonella thypi and probiotic bacteria Lactobacillus plantarum was measured by zone of inhibition. Zone of inhibition by hydrolyzed oils against tested bacteria is presented in Figure 1and 2. Figure 1 and 2 showed that there is difference in bacterial growth. Salmonella thypi grew spread on, while Lactobacillus plantarum grew in colony. This could be due to different characteristic of bacteria. Salmonella thypi is aerobic bacteria while Lactobacillus plantarum is anaerobic one.[11] Fig 1 and 2 showed that tested hydrolyzed oils inactivate tested bacteria. Hydrolyzed oils inhibit bacterial growth with different activity in which Salmonella thypi is more affected than Lactobacillus plantarum indicated by difference in diameter of inhibition zone as presented in Figure 3, 4 and in numerical values in Table 2.

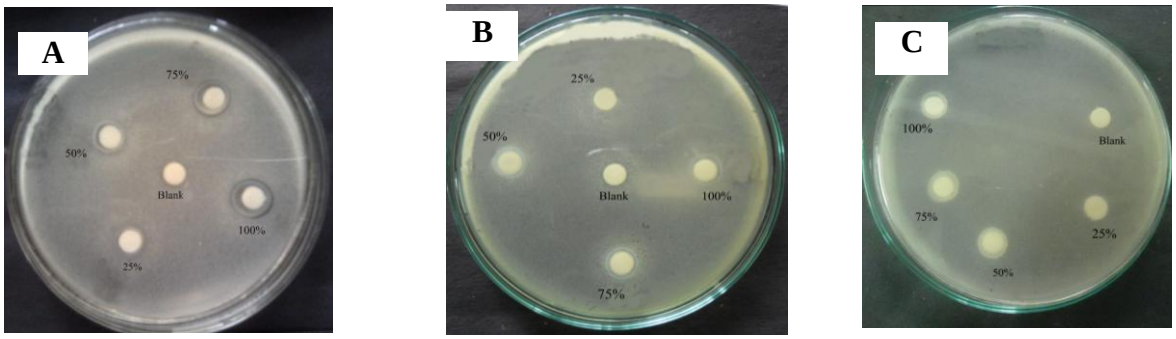

Figure 1. Antibacterial activities indicated by zones of inhibition by hydrolyzed oil on Salmonella thypi. A= Zone of inhibition by hydrolyzed VCO, B= Inhibitory zone of hydrolyzed soybean oil, C= Inhibitory zone of hydrolyzed palm oil

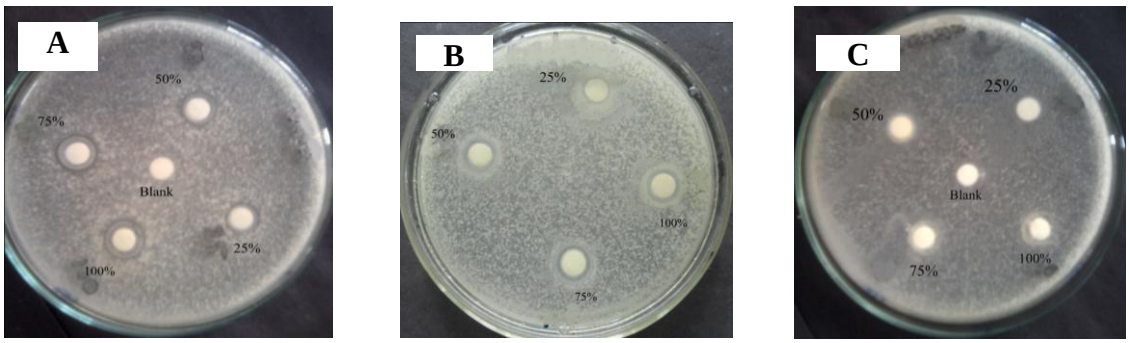

Figure 2. Antibacterial activities indicated by zones of inhibition on Lactobacillus plantarum. A=

Inhibition zone by hydrolyzed VCO, B= Inhibition zone by hydrolyzed soybean oil, C= Inhibition zone by hydrolyzed palm oil

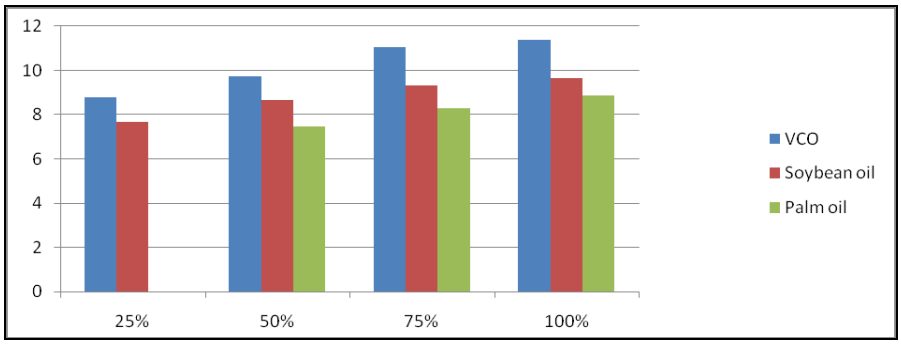

Figure 3. Diameter of Inhibition zone by hydrolyzed oil on *Salmonella typhi*

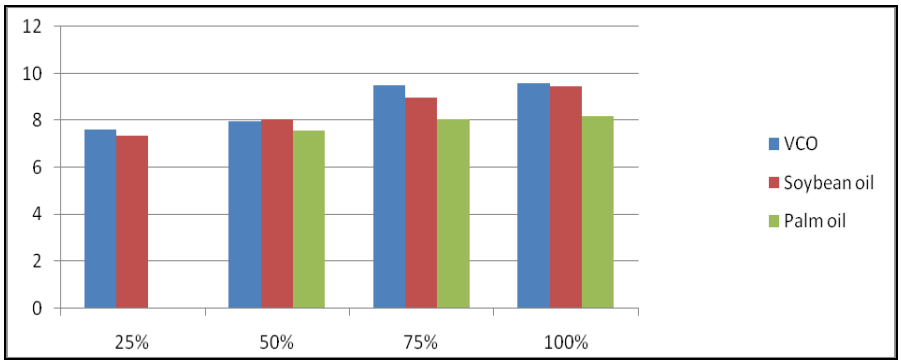

Figure 4. Diameter of Inhibition zone by hydrolyzed on *Lactobacillus plantarum*

Table 2. Diameter of inhibitory zone of hydrolyzed vegetable oil against Salmonella thypi and Lactobacillus plantarum.

Kinds of oil	Concentration (%)	Tested Bacteria	
		Salmonella thypi	*Lactobacillus plantarum*
VCO	25	9,20± 0,50^{c}	7.70± 0,30^{a}
Soybean oil		7,87± 0,15^{e}	7,33± 0,25^{a}
Palm oil		6,00± 0,00*	6,00± 0,00*
VCO	50	9,93± 0,23^{c}	8,00± 0,76^{a}
Soybean oil		8,73± 0,30^{d}	8,00± 0,27^{a}
Palm oil		7,67± 0,40^{e}	7,63± 0,15^{a}
VCO	75	11,10± 0,56^{f}	9,37± 0,85^{b}
Soybean oil		9,50± 0,30^{c}	9,13± 0,23^{b}
Palm oil		8,47± 0,35^{d}	8,13± 0,51^{a}
VCO	100	11,10± 0,92^{f}	9,73± 0,21^{b}
Soybean oil		9,70± 0,27^{c}	9,53± 0,40^{b}
Palm oil		8,57± 0,23^{d}	8,37± 0,31^{a}

Addition: *) diameter of inhibitory zone 6 mm indicated to be non active as antibacterial
Note: Inhibition zones with the same sign are not significantly different (p<0.005).

Antimicrobial activity of an antibacterial material can be classified in to three categories namely (1) active to strong active, (2) moderately active and (3) non active based on the zone of inhibition diameter. From Table 2 can be shown that hydrolyzed VCO at concentration of 25%and 50% is moderately active (6-11 mm), while at concentration 75% and 100% is very active (>11mm) against Salmonella typhi and moderately active against Lactobacillus plantarum at all concentrations. Hydrolyzed soybean oil is moderately active at all concentration against Salmonela typhi and, Lactobacillus plantarum. Hydrolyzed palm oil is non- active (6 mm) at concentration of 25% against Salmonella typhi and Lactobacillus plantarum and is moderately active at concentration of 50%, 75%, and 100% against tested bacteria. [12] From Figure 3, 4 and Table 2 can be seen that hydrolyzed oil found to be active as antibacterial against Salmonella thypi and Lactobacillus plantarum. Antibacterial activity of hydrolyzed oil against both bacteria increases as the concentration of hydrolyzed oil

increases. Hydrolyzed vegetable oils found to have antibacterial activity against both tested bacteria. Antibacterial activity of hydrolyzed oil is higher against *Salmonella thypi* than *Lactobacillus plantarum.* This result was similar compared to previous study, that hydrolyzed oil is more effective against Gram-negative bacteria than Gram-positive bacteria.[3] Hydrolyzed VCO shown to be the highest antibacterial activity against Salmonella typhi and Lactobacillus plantarum, followed by hydrolyzed soybean oil and the lowest antibacterial activity shown by hydrolyzed palm oil. This was due to the difference in fatty acid composition of the hydrolyzed oils. VCO was dominated by lauric acid (about 48%) which was reported as the most active saturated fatty acids and by enzymatic hydrolysis in this study resulted in combination with the most active monoglycerol monolaurine. Soybean oil was dominated by unsaturated long chain fatty acid containing two double bonds linoleic acid (18:2) (about 61%). Palm oil was dominated by saturated long chain fatty acid palmitic acid (about 42.5%) which is less active than unsaturated fatty acids as it was previously reported. [1,3,13]

Conclusion

Hydrolyzed oil is found to be active as antibacterial against Salmonella typhi and Lactobacillus plantarum. Antibacterial activity of hydrolyzed oil is more effective against Salmonella typhi than Lactobacillus plantarum. Antibacterial activity of hydrolyzed oil is affected by length of chain and unsaturation of fatty acid present in the fat molecule. Hydrolyzed VCO which is composed of saturated medium chain fatty acid especially lauric acid has the highest antibacterial activity, followed by hydrolyzed soybean oil composed of unsaturated long chain fatty acid and the lowest is by hydrolyzed palm oil composed of saturated long chain fatty acid.

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