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Anti Bacteria Activities of Lauric Acid from Coconut Endosperm (Hydolysed using lipase Endogeneus)

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ABSTRACT

Background: Lauric acid is very useful as an anti-bacterial, Coconut oil contains lauric acid 48%. **Objectives:** This research aims to study activity of lauric acid as an anti-bacterial activity of pathogenic and spoilage. Lauric acid can be isolated from coconut endosperm by the method of hydrolysis using lipase endogeneus. Research using experimental methods. Media containing bacterial pathogens (*Salmonella sp., E. Coli*, and *Stafilococus aureus*) and spoilage bacteria (*Micrococcus*, *Pseudomonas* and *Bacillus stearothermophilus*) was added lauric acid with varying concentrations (0-75%). Then, determinated Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MKC). **Results:** The results showed that lauric acid can inhibit (MIC) *Salmonella sp., E. Coli, and Stafilococus aureus* at concentrations of 3.13%. As against *Micrococcus* at a concentration of 10%, *Bacillus stearothermophilus* at a concentration of 30% and *Pseudomonas* at concentrations of 50%. MKC lauric acid against Salmonella amounted to 3.13%, while *E. coli* and *Staphilococus aureus* 6.25%. MKC against *Micrococus* at a concentration of 30%, *Bacillus stearothermophillus* at concentrations of 50% and *Pseudomonas* at concentrations of 70%. **Conclusion:** Lauric acid was isolated from coconut endosperm can be killed bacterial pathogenic (*Salmonella* bacteria, *Stafilococus aureus* and *E. Coli*) with concentration lower than spoilage bacterial (*Micrococus*, *Bacillus stearothermophillus* and *Pseudomonas*).

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INTRODUCTION

Lauric acid and medium-chain fatty acids (MCFA) as capric acid and myristic acid is very useful as an anti-bacterial [10], can inhibit the development of the HIV virus [2], herpes virus, influenza and sarcoma [7]. In addition, lauric acid can lower blood cholesterol levels [5]

Kabara *et al.* [4] states that, lauric acid and other fatty acids such as capric acid, palmitic acid, myristic acid, linoleic acid, linolenic acid can inhibit the growth of *pneumococci, streptococci, Micrococci, Candida, S. aureus, S. epidermis.* Lauric acid concentration requires only 0.062 micro mol / ml was able to inhibit pneumococci. While capric acid and myristic acid each requires 1,45 micro mol/ml and 0,218 micro mol/ml to inhibit pneumococci.

Coconut oil contains lauric acid 48% [3]. Meanwhile, according [8] coconut oil contains lauric acid 51-53%.

Lauric acid can be isolated from coconut oil by breaking the ester bond in coconut oil so that regardless of lauric acid glycerol. Termination of the ester bond in coconut oil can be done by several methods including methods methanolyisis, hydrolysis and saponification. Methanolyisis method involves reacting coconut oil with methanol using a catalyst NaOCH3. Method of saponification carried out with the addition of bergliserol soda (NaOH mixture with glycerol). Hydrolysis method was conducted by heating at high temperature and pressure or by enzymatic methods [1].

Enzymatic hydrolysis has been carried out by [9], which uses endogenous lipase coconuts. Coconut destroyed by adding water as much as 100% of the weight of coconut endosperm. Hydrolysis is carried out at 35 ° C for 72 hours using lipase endogeneus in the coconut with a specific activity of 1.82 units / mg protein. Lauric acid fraction produced as much as 48.25% of oil in the substrate. Lauric acid fraction contains lauric acid

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content of 53.86% and the rest of myristic acid, capric acid, palmitic acid and other fatty acids. The amount of lauric acid resulting from the hydrolysis of 25.86% of total coconut oil [9].

Lauric acid that isolated from coconut oil by enzymatic methods have not tested the ability of anti-microbial. Therefore, it is necessary to reseach the ability of anti-microbial of lauric acid isolated from coconuts with enzymatic method. This research studied how the minimum concentration required of lauric acid to kill some microbes (pathogens and spoilage)

Methodology:

This study was conducted from April to October 2014 in the Laboratory of Chemistry and Biochemistry University of Widya Gama Malang. Several stages of research conducted at the Laboratory of Microbiology University of Brawijaya Malang, laboratories of microbiology University of Muhammadiayh Malang, and laboratories PAU University of Gadjah Mada.

The tools used in this study includes a set of tools glasses, micro pipette, plastic filters, blade stailess steel, stainless steel grate tool (Brilliant), mortar, centrifuges, analytical balance (Mettler Toledo AL 204), magnetic stirrer (stirrer), heating (Janke-Kunkel), oven, pH-meter (Orion 201), room thermometer, UV-Vis spectrophotometer (Genesys 10 UV series), gas chromatography (model HP 5890 series) with CBPS column.

Materials used, among others, the coconut varieties of dalam from Lawang Malang, virgin coconut oil (VCO) brand Bagoes, aquades, deionized distilled water. Chemicals, among others, gum arabic, ammonium sulfate salt, NaOH, pp indicators, K2HPO4, KH2PO4, CuSO4, KNa-tartrate, HCl, petroleum ether, diethyl ether, distilled water, ethanol, Na2CO3, twin, agar nutrien.

Implementation of Research:
This research was conducted in two phases:
Stage 1 : Isolation of lauric acid from coconut endosperm
Stage 2 : Test of antibacterial activity lauric acid

***Lauric acid isolation** ([1] ; [14]):*

Coconut (old) peeled husk fiber and then shredded . Grated coconut added water (1 : 1) then squeezed to obtain coconut milk . Coconut milk further hydrolyzed using lipase . Lipase using endogenous enzymes contained in the coconut milk (endogenous) .

A mixture of substrate (coconut milk) with a lipase enzyme then hydrolysed at a temperature of 35 ° C with for 72 hours. Hydrolysis products separated the free fatty acid fraction from gliseride fractions . The free fatty acid fraction is the fraction of lauric acid to be tested levels of lauric acid and tested the ability of anti-bacterial .

Free fatty acids (FFA) were separated from gliseride fraction using Mattick and Lee (1959) . Lauric acid assay using Gas Chromatography (GC) .

Determination of Lauric acid activity as an anti- bacterial [4]:

Anti-bacterial test lauric acid fraction includes testing the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MKC) using a dilution method.

The study was conducted by adding lauric acid fraction obtained from the research phase 1 (different concentrations) to the samples containing microorganisms (*Micrococci , Stafilococus aureus , Salmonella , E. coli , Pseudomonas* and *Bacillus stearothermofilus*) 10^7 cfu. Samples were then incubated at 35 ° C for 24 hours and then counted the number of bacteria

RESULTS AND DISCUSSION

Stage 1: Lauric Acid Isolation:

Lauric acid fraction isolated from coconut milk after dihirolisis for 72 hours. Lauric acid fractions obtainedd for 48.98% of the amount of oil in the coconut milk. The highest fatty acid in lauric acid was lauric acid so-called lauric acid fraction. The levels of lauric acid in the fractions were 50.45%.

Stage 2: Test Anti Bacteria:
a. Minimum Inhibitory Concentration (MIC):

Minimum Inhibitory Concentration (MIC) is the minimum concentration of a compound which can inhibit the growth of (mostly) microbes from the initial microbial count. The Minimum Inhibitory Concentration (MIC) of lauric acid fractions were tested for bacterial pathogens include *Salmonella, Stafilococus aureus* and *E. coli* and non-pathogenic bacteria (bacterial decay) was *Micrococci, Pseudomonas* and *Bacillus stearothermofilus*.

MIC test results showed that the concentration of lauric acid fraction 3.13% (equivalent to 3.13 g / 100 ml or 31.3 mg / ml) is able to inhibit the growth of bacteria *Salmonella, Stafilococus aureus* and *E. coli*. Minimum

Inhibitory Concentration (MIC) test for bacteria spoilage showed that the fraction of lauric acid was only able to inhibit the growth of bacteria at a concentration of 10 % *Micrococcus* , *Bacillus stearothermophillus* at a concentration of 30 % and *Pseudomonas* at concentrations of 50 %. The results showed that the most easy *Micrococcus* inhibited by lauric acid , whereas *Pseudomonas* was the most difficult bacteria growth was inhibited by lauric acid . Results can be seen in Table 1.

Table 1: Minimum Inhibitory Concentration (MIC) of Lauric acid (%) to Inhibition of pathogenic bacterial and spoilage bacteriall.

Kinds of Bacteria	Minimum Inhibitory Concentration (MIC) of Lauric acid (%)
Pathogen bacteria	
Salmonella	3.13
E. coli	3.13
Stafilococus aureus	3.13
Spoilage bacteria	
Pseudomonas	50.00
Micrococcus	10.00
Bacillus strearothermophilus	30.00

b . Minimum Kill Concentration (MKC):

Minimum Kill Concentration (MKC) is the minimum concentration of a compound that can kill all microbes or to the remaining maximum of 0.01 % of the initial amount .

Lauric acid fraction has have a different MKC for pathogenic bacteria *Salmonella, Stafilococus aureus* and *E. Coli* . On *Salmonella* , requires lauric acid concentrations lower than *Stafilococus aureus* and *E. Coli* . Lauric acid concentrations were 3.13% to kill the bacteria *Salmonella*. As for killing *Stafilococus aureus* and *E. coli* requires a concentration of 6.25% lauric acid .

Minimum Kill Concentration (MKC) Test Results showed that lauric acid fraction can be killed spoilage bacteria Bacillus stearothermophillus for 50 %, Micrococus bacteria for 30% and Pseudomonas for 70 % of concentrations. The results of MKC test for pathogenic and spoilage bacteria can be seen in Table 2 The results of MKC test for pathogenic and spoilage bacteria can be seen in Table 3 and Figure 1-3 .

Table 2: Minimum Kill Concentration (MKC) of Lauric acid (%) to Inhibition of pathogenic bacterial and spoilage bacteriall.

Kinds of Bacteria	Minimum Kill Concentration (MIC) of Lauric acid (%)
Pathogen bacteria	
Salmonella	3.13
E. coli	6.25
Stafilococus aureus	6.25
Spoilage bacteria	
Pseudomonas	70.00
Micrococcus	30.00
Bacillus strearothermophilus	50.00

Research results [4] showed that lauric acid which is already pure (lauric acid content of at least 99%) were able to inhibit the *Stafilococus aureus* bacteria (pathogens) at a concentration of 2.49 micromol / ml (equivalent to 0.4283 mg / ml). This shows that lauric acid obtained from the hydrolysis of coconut endosperm has the ability to inhibit bacterial lower than with previous research lauric acid. This is because the purity levels of lauric acid from coconut is still low at 53.86%.

Mono acyl glycerol (mono laurin) obtained from coconut oil inhibited *Stafilococcus aureus* and *Salmonella* bacteria have been carried out by [6]. The results showed that the mono laurin from coconut oil could inhibit *Stafilococcus aureus* and *Salmonella* bacteria at a concentration of 12.5 mg / ml. This shows that the mono laurin of coconut oil has a higher inhibitory power of the lauric acid from coconut oil. This is consistent with the results of [4] which states that the inhibition of mono laurin higher than in lauric acid against bacteria.

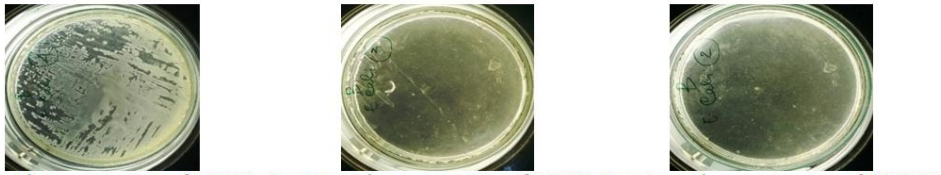
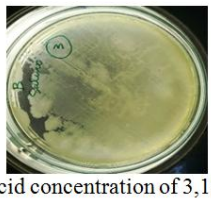


Fig. 1: The number of bacteria *E. coli* the addition of different concentrations of lauric acid.


Lauric acid concentration of 3,13%


Lauric acid concentration of 6,25%

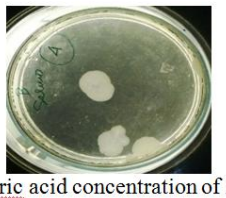

Lauric acid concentration of 2,50%

Fig. 2: The number of bacteria *Salmonella* the addition of different concentrations of lauric acid.


Lauric acid concentration of 3,13%


Lauric acid concentration of 6,25%

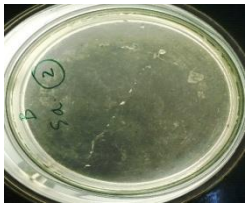
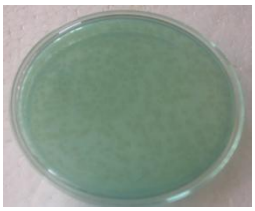
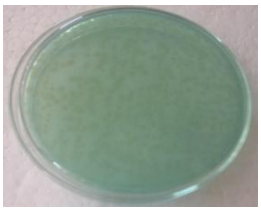

Lauric acid concentration of 12,50%

Fig. 3: The number of bacteria *Stafilococus aureus* the addition of different concentrations of lauric acid.


Lauric acid concentration of 55%


Lauric acid concentration of 60%

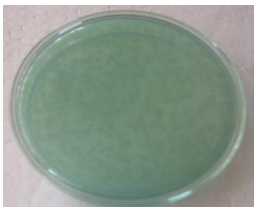
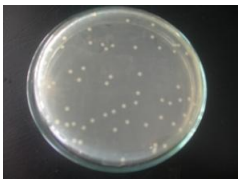
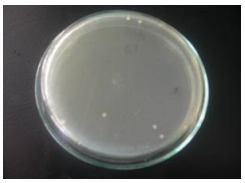

Lauric acid concentration of 65%

Fig. 4: The number of bacteria *Pseudomonas* the addition of different concentrations of lauric acid.


Lauric acid concentration of 20%


Lauric acid concentration of 25%

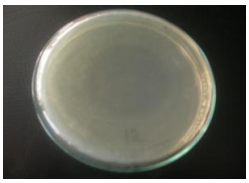
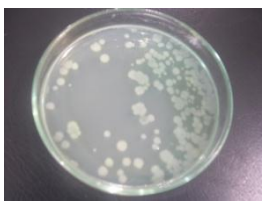
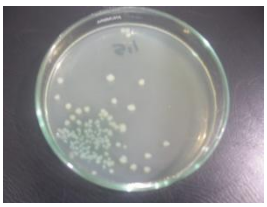

Lauric acid concentration of 30%

Fig. 5: The number of bacteria *Micrococus* the addition of different concentrations of lauric acid.

The ability of lauric acid fraction in killing spoilage bacteria is lower compared against pathogenic bacteria. For pathogenic bacteria with a concentration of 3.13% lauric acid have been able to kill the bacteria *Salmonella* and 6.25% concentrations are able to kill *Stafilococus aureus* and *E. coli*. The concentration of 6.25% lauric acid was still not able to kill the spoilage bacteria. Lauric acid fraction can kill bacteria *Micrococus* at 30% concentration, the bacteria *Bacillus stearothermophillus* at a concentration of 50%, and *Pseudomonas* at concentrations of 70%.

Bacterial pathogens more easily inhibited growth by lauric acid compared spoilage bacteria (non-pathogenic). This was because the pathogens more easily disabled than spoilage bacteria.


Lauric acid concentration of 40%


Lauric acid concentration of 45%

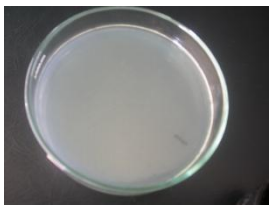

Lauric acid concentration of 50%

Fig. 6: The number of bacteria *Bacillus strearothermophilus* the addition of different concentrations of lauric acid.

Conclusion:

a. Conclusion:

Lauric acid can be killed Salmonella bacteria at a concentration of 3.13% , *Stafilococus aureus* at 6.25% and *E. Coli* at 6.25%. Lauric acid was able to kill bacteria *Micrococus* at a concentration of 30% , *Bacillus stearothermophillus* at concentrations of 50 % and *Pseudomonas* at concentrations of 70 % .

b . Suggestion:

Further research can be to study the ability of anti-bacterial lauric acid for animal experiments.

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