International Journal of Engineering & Technology, 8 (1.9) (2019) 339-342

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Fractinations of Coconut Oil from Coconut Milk and Antibacterial Activities

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Abstract

Hydrolysed coconut oil contained of free fatty acid (FFA fraction), monoglyceride, diglycerides, and triglycerides (glycerides fraction). Free fatty acid (lauric acid, caprylic acid, capric acid) and monoglyceride (monolaurin, monocaprin, etc) had antibacterial activity. Triglycerides and diglycerides had none antibacterial activity. This research was studied about which fraction that had most excellent antibacterial activity. It was prepared in two stages : the first step was fractination of coconut oil and the second step was tested of antibacterial activity. Antibacterial test were carried out with dilution method. The media contained of bacteria (Bacillus stearothermophillus, Salmonella sp., E. Coli, and Staphylococus aureus) was added by coconut oil fractions (unhydrolysed coconut oil, hydrolysed coconut oil, free fatty acids and glycerides fraction) with varying concentrations (0 to 12.5%). All fractions were tested antibacterial activity against Bacillus stearothermophilus. Furthermore, only two fractions (free fatty acids and glycerides fraction) were tested antibacterial activity against Salmonella sp., E. Coli, and Staphylococus aureus bacteria. The results revealed that free fatty acids fraction with 6.25% (w/v coconut oil) could inhibit Bacillus stearothermophilus bacteria. While, unhydrolysed coconut oil, hydrolysed coconut oil and glycerides fraction could not able to inhibit the Bacillus stearothermophilus bacteria up to 12.5%. Free fatty acids and glycerides fractions with 3.13% (w/v coconut oil) could inhibit Salmonella sp. and Staphylococcus aureus bacteria. Whereas, the E. Coli bacteria could be inhibited by free fatty acids fraction with 3.13% (w/v coconut oil) and glycerides fraction with 6.25% (w/v coconut oil).

Keywords: *Product design, QFD TRIZ, DFMA.*

1. Introduction

Long-term program (2015-2025) of the Directorate General of Agro and Chemical Industrial Department in 2009, coconut processing is directed to coco-chemical products development and the development of downstream industry from coco-chemical products (Industrial Department, 2009).

Coconut oil contain some of fatty acids that included caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearat acid, oleic acid and linoleic acid. Lauric acid is the highest fatty acid of 52.26%, followed by myristic acid of 16.82% and caprylic acid of 8.21%. Then, capric acid of 7.79%, caproic acid of 0.24%, palmitic acid of 6.59%, stearic acid of 1.51%, oleic acid of 4.83%, linoleic acid of 1.33% (Su'i, 2009).

There are two kinds of coconut oil were unhydrolysed coconut oil and hydrolysed coconut oil. Hydrolysed coconut oil was processed from hydrolysis of coconut milk, and then processed to obtain coconut oil. Unhydrolysed coconut oil was processed from fresh coconut milk without hydrolyzing process.

Lipase enzyme is an enzyme which able to hydrolyze ester bond, especially neutral lipid like triglyceride (Sana et al., 2001). Lipase enzyme has hydrolysis ability, that was separating oil into free fatty acid and glycerol (Pahoja et. al., 2001).

Lipase enzymes endogenous from coconuts can hydrolyze coconut oil. Lipase enzymes in the coconut milk had the specific activity 1.82 units/mg of protein. The result of hydrolyzing that obtained from free fatty acid was 0.15 ml mol/ml of coconut milk or 0.23 ml mol/g of coconut (Su'i et. al., 2014).

Several fatty acids from coconut oil that hydrolyzed will released becomes free fatty acids. Partial fatty acids could not hydrolysed and still bounded with glycerol. The fatty acids that was still bounded with glycerol can be a diglyceride, monoglyceride, or triglycerides.

Free fatty acid from hydrolysis product can be separated from unfree fatty acids fraction so that the free fatty acid value is higher. The residue of the separation is glycerides fraction which still contain of monoglycerides, diglycerides, and triglycerides. Therefore, the research need to know the antibacterial activity of unhydrolysed and hydrolysed of coconut oil with varying fractionation results.

Medium chain fatty acids (MCFA) such as lauric acid, capric acid and myristic acid is very useful as an antibacterial (Vetter and Schlievert, 2005). Beside that, lauric acid can inhibit the development of the HIV virus (Conrado, 2002), the herpes virus, influenza and sarcoma (Preuss, 2001). Lauric acid can lower the blood cholesterol levels (Nicole, Evert and Martijn, 2001).

The growth of several bacteria such as Pneumococci, Streptococci, Micrococci, Candida, S. aureus, S. epidermis were inhibited by lauric acid, capric acid, palmitic acid, myristic acid, linoleic acid, linolenic acid (Kabara et al., 1972).

Fatty acids had antibacterial ability if in form of free fatty acids and monoglycerides. Lauric acid require 0.62 micromoles/ml to inhibit Micrococci bacterial. Meanwhile, monolaurin require 0.09 micromoles/ml (Kabara et al., 1972).

Fatty acids which in diglycerides and triglycerides forms, had none antibacterial activity. Lauric acid in form of tri laurin and dilaurin could not inhibit the growth of Micrococci up to 6 micromoles/ml. While, capric acid require 2.90 micromoles/ml and monokaprin require 0.10 micromoles/ml to inhibit Micrrococci. Meanwhile, dikaprin could not able to inhibit Micrococci (Kabara et al., 1972).

Free fatty acids were obtained by releasing fatty acids from coconut oil ester bonds. The process was carried out with several methods included methanolysis, hydrolysis and saponification (Alamsyah and Nuryanti, 2004).

This research was studied about which fraction that had most excellent anti-bacterial activity from coconut oil.

2. Methodology

This research was carried out in two stage. Stage 1: fractination of coconut oil. Stage 2: Antibacterial activity test of coconut oil with varying fractions.

2.1. Hydrolysis Process (Su'i et al., 2014)

Coconut (old) was peeled and the epidermis was grated. Then, added water 100% from coconut weight (1: 1) to make coconut milk. After that, coconut milk was hydrolysed using lipase enzyme that taken from endogenous enzymes in the coconut milk (endogenous). The mixture of substrate (coconut milk) with lipase enzyme were hydrolysed for 72 hours at 35 oC.

2.2. Fractination

Hydrolysis products were divided into two parts. The first part was sentrifuged for 20 minutes with 3000 rpm in order to obtain two fractions, oil fraction (top) and non-oil fractions (below). Then, oil fraction was taken carefully by pipette separated from non-oil fraction. Oil fraction was namely hydrolysed coconut oil (Su'i et al., 2014).

The second part was separating free fatty acids fraction and glycerides fraction. The separation of free fatty acids (FFA) by using Mattick and Lee methods (1959). This part was obtained free fatty acid fraction and glycerides fraction.

Meanwhile, unhydrolysed coconut oil fraction was obtained in the same way, but did not used hydrolysis. Coconut was grated and added with water 1: 1 in order to obtain coconut milk. Furthermore, coconut milk was freezed for 12 hours. The next step was placed at room temperature (25° C) and centrifuged for 20 min at 3000 rpm to obtain two fractions, oil and non-oil fraction. Oil fraction were separated to obtain unhydrolysed oil (Su'i et al., 2014).

All fractions were measured yield, free fatty acid and lauric acid.

2.3. Antibacterial Activity Test (Kabara et al., 1972).

Minimum Inhibitory Concentration (MIC) of coconut oil fractions was measured by dilution method.

Antibacterial test was carried out in two stages. The first, evaluating antibacterial activity in all fractions (unhydrolysed oil, hydrolysed oil, free fatty acids fraction and glycerides fraction) with Bacillus stearothermophyllus. The second, free fatty acids fraction and glycerides fraction were evaluated antibacterial activity to against pathogenic bacteria (Staphylococcus aureus, Salmonella and E. coli).

The evaluation was conducted by adding coconut oil fraction with concentration of 0-12% in agar medium that containing bacteria (Staphylococcus aureus, Salmonella, E. coli, and Bacillus stearothermophyllus) 107/ml. Then, Sample was incubated at 35°C for 24 hours and observed the growth of bacteria.

3. Discussion and Conclusion

3.1. Stage I. Fractination

3.1.1. The yield

The results revealed that unhydrolysed and hydrolysed oil fraction yields were higher than free fatty acids and glycerides fraction. The yield of coconut oil fractionation were shown in Table 1.

Table 1: The yield of coconut oil fractination from coconut milk

Fractions	Yield (%)
Unhydrolysed Oil	11.70
Hydrolysed Oil	11.40
Free Fatty Acid Fraction	5.50
Glyserides Fraction	5.90

The yield of free fatty acid and glycerides fraction were lower than unhydrolysed and hydrolysed oil fraction. In this case, free fatty acid and glycerides fraction were obtained from separation of hydrolysed coconut oil fraction. Coconut milk was hydrolysed for 72 hours, and then was extracted to get hydrolysed oil fractions. Finally, hydrolysed oil was fractinated using an organic solvent to separate into two fractions, free fatty acids (below) and glycerides (above).

3.1.2. Free fatty acid content

The value of free fatty acids was 0.60 mmol/ml in unhydrolysed coconut oil and 1.32 mmol/ml in hydrolysed oil fractions. While, free fatty acid and glycerides values were not be observed with assumed that almost all free fatty acid fraction (100%) in free fatty acids form. Beside, there was no free fatty acid (0%) in glycerides fraction. In separation process, all free fatty acids would be at the bottom and glycerides at the top. Free fatty acid values could be seen in Table 2.

Table 2: Value of Free Fatty Acids in the Coconut Oil Fractions

Fractions	Free Fatty Acids (m mol/ml oil)
Unhydrolysed Oil	0,60
Hydrolysed Oil	1,32
Free Fatty Acids Fraction	Not measured [1)]
Glyserides Fraction	Not measured [2)]

Note : 1) Assumed in approach 100 % ; 2) assumed no free fatty acids

Free fatty acid values in the hydrolysed oil was higher than unhydrolysed oil. it was caused coconut oil was hydrolysed by lipase enzymes and produced free fatty acids. Tambun (2002) stated that endogeneus lipase enzyme able to hydrolyse palm oil into free fatty acids.

Endosperm lipase enzyme from coconut can hydrolyse coconut milk into free fatty acids. The longer hydrolysis, free fatty acid values will increased (Su'i et al., 2014).

Lipase enzyme can hydrolyse fat or oil into free fatty acids. Lipase enzyme will release fatty acid with glycerol bonds at position 1 or 2 (Sana et al., 2004).

3.1.3. Fatty acid composition

Fatty acid composition was only observed in unhydrolysed, hydrolysed oil and free fatty acid fraction. Glycerides was assumed no free fatty acids in that fraction because it was residue from separation of free fatty acid fraction from coconut oil in coconut milk. Lauric acid value was the highest in all fractions with varying amounts. Fatty acid composition could be seen in Table 3.

Table 3: Fatty Acid Composition in Unhydrolysed Oil, Hydrolysed Oil and Free Fatty Acid Fraction

Fatty acid	Free Fatty Acid (% b/v)		
	Unhydrolysed Oil Fraction	Hydrolysed Oil Fraction	Free Fatty Acid Fraction
Kaproic	0,00	0.00	0.71
Kaprilic	0.25	2.98	7.86
Kapric	0.31	3.73	7.76
Lauric	2.28	25.86	53.86
Myristic	0.79	8.63	16.71
Palmitic	0.32	3.47	6.60
Linoleic	0.21	2.43	4.38
Oleic	0.11	1.15	2.12

Lauric acid was the highest in all fractinated coconut oil. It was caused lauric acid value in coconut oil was the highest. Su'i (2009) stated that lauric acid in coconut oil was highest than other fatty acids, lauric acid 52.26%, followed by myristic acid 16.82%, caprylic acid 8.21%, and capric acid 7.79%. As composition, lipase enzyme had the highest chance to hydrolyze lauric acid than other fatty acids. Thus, the amount of lauric acid was the highest than other after hydrolysis.

3.2. Stage II. Anti-Bacterial Activity

3.2.1. Bacillus Stearothermophylus

Free fatty acid fraction could inhibit the growth of Bacillus Stearothermophylus bacteria at 6.25% (w/v coconut oil). While, unhydrolysed oil, hydrolysed oil and glycerides fraction could not inhibit Bacillus Stearothermophylus growth until 12.50%. For comparison, ampicillin at 0.3 mg/100 ml could inhibit the growth of bacteria. Inhibitory ability of coconut oil fractions against Bacillus Stearothermophylus bacteria could be seen in Table 4.

Table 4: Minimum concentration of coconut oil fractions and ampicillin against the growth of Bacillus Stearothermophylus bacteria

Sample	Minimum Inhibitory Concentration (MIC)
Unhydrolysed Oil	NI
Hydrolysed Oil	NI
Free Fatty Acids Fractions	6,25 %
Glyserides Fractions	NI
Ampisilin	0,30 mg/100 ml

NI : Non Inhibition Bacillus Stearothermophylus up to 12,5% pf sample.

Free fatty acid fraction is the highest antibacterial activity than others. It was caused lauric acid value in free fatty acid fraction was higher than other fractions. Free fatty acid fraction containing 53.86% lauric acid and hydrolysed oil only 25.86% (Table 3).

3.2.2. Pathogens bacteria

Antibacterial test at this stage only evaluating free fatty acids and glycerides fraction. Pathogenic bacteria that were used such as Salmonella, E. Coli and Staphylococcus Aureus. Free fatty acids and glycerides fraction can inhibit the growth of Salmonella, E. Coli and

Staphylococcus Aureus with varying concentrations. Inhibition ability of free fatty acids and glycerides fraction against Salmonella, E. Coli and Staphylococcus Aureus could be seen in Table 5.

Free fatty acid fraction with 3.13% (w/v coconut oil) could inhibit the growth of Salmonella, E. Coli and Staphylococus Aureus. While, antibacterial activity from glycerides fraction was lower to against E. Coli. Glycerides fraction could inhibit E. coli at 6.25%, then Staphylococcus Aureus and Salmonella at 3.13%.

Free fatty acids fraction is most excellent to inhibit the growth of bacteria because it is containing lauric acid, myristic acid, caprylac acid and caprylic acid. According to (Kabara et al., 1972), lauric acid could inhibit Staphylococcus aureus growth at 2.49 micromoles/ml (equivalent to 0.4283 mg/ml).

Glycerides fraction also can inhibit the growth of bacteria. It assumed that glycerides fraction is containing monoglycerides (mono laurin, miristin mono, mono kaprin, mono kaprilin). (Kabara et al., 1972) stated that laurin mono, mono kaprin could inhibit bacteria growth.

Mono laurin from coconut oil was able to inhibit the growth of Staphylococcus aureus and Salmonella at 12.5 mg/ml (Nuraida et al., 2008).

Table 5: Inhibition of Coconut Oil Fractions in Some Bacteria

Sampel Concentr.	Salmonella		E. Coli		S. Aureus	
	Gliseri	FFA	Gliseri	FFA	Gliseri	FFA
0.00%	NI	NI	NI	NI	NI	NI
0.195%	NI	NI	NI	NI	NI	NI
0.39%	NI	NI	NI	NI	NI	NI
0.78%	NI	NI	NI	NI	NI	NI
1.56%	NI	NI	NI	NI	NI	NI
3.13%	I	I	NI	I	I	I
6.25%	I	I	I	I	I	I
12.50%	I	I	I	I	I	I

Note : Gliseri : Gliseride fraction

FFA : Free Fatty Acid fraction

Conclusion from this research, most excellent fraction to inhibit the growth of pathogen bacteria (Bacillus stearothermophillus, Salmonella, Staphylococus aureus, and E. Coli) is free fatty acid fraction. Free fatty acid fraction can inhibit the growth of Bacillus stearothermophillus at 6.25% (w/v). Meanwhile, glycerides fraction, hydrolysed and unhydrolysed oil fraction were not able to inhibit that bacteria up to 12,5%.

Inhibition of growing Bacillus stearothermophillus bacteria require the higher free fatty acid concentration than other pathogenic bacteria (Salmonella, Staphylococus aureus, and E. Coli).

Free fatty acids fraction with 3.13% can inhibit the growth of Salmonella, Staphylococus aureus, and E. Coli bacteria. Then, glycerides fractions require 3,13% to inhibit the growth of Salmonella, Staphylococus aureus, and 6,25% for E. Coli.

Acknowledgement

The authors are grateful thanks to the Directorate General of Education for providing financial during investigation through 2016 Research Grant Program.

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