THE MODIFICATION OF RED PALM OIL AND PALM KERNEL OIL AS ANTIBACTERIAL LIQUID SOAP

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ABSTRACT
Triglyceride and strong base are the main constituents to form fatty acid salts. These could be obtained from refined red palm oil (RPO) and palm kernel oil (PKO). RPO is a derivative product of palm oil which contains high levels of squalene, vitamin E, and carotenoids. These compounds are a source of natural nutrients for skin health. Meanwhile, PKO is rich in lauric acid that can kill acne-causing bacteria such as Propionibacterium acnes and Staphylococcus epidermidis. This research was conducted to prepare and evaluated the antibacterial activity of the fatty acid salts of RPO and PKO. RPO and PKO were mixed with potassium hydroxide to form fatty acid salts. The antibacterial activities of fatty acid salts to inhibit Propionibacterium acnes and Staphylococcus epidermidis were evaluated using the method. The fatty acid salts at concentrations of 10%, 20%, 30%, 40%, and 50% were tested for their activities against fat acid bacteria. The optimum activity of the fatty acid salts was formulated for face cleanser liquid soap. The physical stability was then evaluated for six days by using the cycling test method. The fatty acid salts of RPO: PKO (50:50) with 30% concentration showed the optimum activity; thus, it was formulated as a face cleanser liquid soap. The stability evaluation showed that the soap complied with Indonesian National Standard No. 06-4085-1996. The fatty acid salts of RPO: PKO (50:50) may be formulated as nutritional liquid soap with optimum antibacterial activity.

Keywords: Red Palm Oil, Palm Kernel Oil, Fatty Acid Metal Salts, Antibacterial, Liquid Soap

INTRODUCTION
Bacteria Propionibacterium acnes and Staphylococcus epidermidis cause skin acne.¹ Hard and liquid soap can clean and protect facial skin from these bacterial infections and prevent skin from infectious diseases.² Cleansing products such as liquid face cleanser generally use sodium laurel sulfate (SLS) as detergent, which acts as an anionic surfactant.³ However, high SLS concentration harms the eyes, causing acute irritation and blindness.⁴ Fatty acid salts are an option as a cleaning agent because it has hydrophilic and lipophilic groups. Fatty acid salts can be formed from the reaction of oils/fats with strong alkaline, which is known as saponification.⁵ Palm oil and palm kernel oil are types of oil that can be used as a source of fatty acid salts. Red palm oil (RPO) is a derivative product of palm oil through the refining process without heating and bleaching.⁶ RPO is rich in unsaturated fatty acids such as oleic, linoleic, and linolenic acid whereas PKO is rich in saturated fatty acids such as lauric acid.⁷ There are some minor components in RPO, such as carotenoid, vitamin E, and squalene, that act as antioxidants in palm oil.⁷,⁸ These antioxidants are natural antioxidants that are used in a topical formulation. Vitamin E, squalene and carotene have important parts in protecting the bio-membrane from peroxidation, protecting skin from sunburn, maintaining skin moisture and also enhancing body immune.⁹,¹⁰ This study aims to produce a liquid facial soap formula using fatty acid salts with a combination of RPO and PKO, which is useful as an antibacterial agent for Propionibacterium acnes and Staphylococcus epidermidis.

EXPERIMENTAL
Fatty Acid Salts Preparation
Red palm oil was mixed with palm kernel oil with a ratio of 100: 0, 80:20, 50:50, 20:80, and 100: 0 (w/w) at 70ºC for 30 minutes. The mixed palm oil and palm kernel oil are added with potassium hydroxide at a temperature of 60ºC for 30 minutes to produce a transparent solid form and heated to balanced pH.¹⁰
Antibacterial Activity Evaluation of Fatty Acid Salts
Fatty acid salts were diluted with water to produce the following concentration by 10, 20, 30, 40, and 50%. About 0.1 ml of bacteria suspension was brought into a petri dish. Melted 20 ml sterile gelatin nutrients were added. The mixture was blended until homogenous and condensed. The petri dish was then soaked in the modified fatty acid solution, placed on the gelatin media’s surface, and followed by incubation for 18 - 24 hours in temperature 35 ± 2°C. The inhibitor diameter zone around the petri dish was measured, and the test was conducted triplicate. The best concentration of fatty acid salts was used as the formulation of antibacterial liquid soap preparation.

Liquid Soap Formulation
The most optimum variation of fatty acid salts in the previous concentration was then chosen to be formulated as a face cleanser liquid soap preparation. The basic formulation of liquid soap formulation is by diluting water into fatty acid salts. The previous thick liquid formulation was added using a variable concentration of liquid carboxomer of 1%, 3%, and 5%.

Physical Evaluation
The liquid soap preparation was evaluated based on the organoleptic, pH, specific weight, free alkaline number, total numbers of the plate, foam weight, and viscosity.

Stability Test
The stability test of three liquid formulations was carried out by using a freeze-thaw cycling test with slight modification. The liquid soap stability test was performed by keeping the formulation between 4°C, 25°C, and 40°C, each for 24 hours in 6 cycles.

Irritation Test
The irritation test of liquid soap formulation was performed on 12 volunteers to determine the itch, redness, or skin coarsely. The method used in this irritation test was an open test; performed by applying the sample three times a day for two days. The possible positive reaction of irritation is itch, redness, or coarse skin on the tested area.

RESULTS AND DISCUSSION
Antibacterial Activity of Fatty Acid Salts
The result of fatty acid salts antibacterial activity for Propionibacterium acne and Staphylococcus epidermidis was presented in Table-1 and Table-2.

Table-1: Antibacterial Activity Test of Fatty Acid Salts to Propionibacterium acne

<table>
<thead>
<tr>
<th>Fatty Acid Salts</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Palm Oil</td>
<td>8.33 ± 0.15</td>
<td>-</td>
<td>9.33 ± 0.20</td>
<td>-</td>
<td>10.26 ± 0.20</td>
</tr>
<tr>
<td>RPO with PKO 20%</td>
<td>8.33 ± 0.15</td>
<td>9.33 ± 0.20</td>
<td>10.26 ± 0.20</td>
<td>10.53 ± 0.25</td>
<td>11.73 ± 0.15</td>
</tr>
<tr>
<td>RPO with PKO 50%</td>
<td>18.03 ± 0.11</td>
<td>18.30 ± 0.10</td>
<td>20.23 ± 0.05</td>
<td>20.46 ± 0.15</td>
<td>20.66 ± 0.15</td>
</tr>
<tr>
<td>RPO with PKO 80%</td>
<td>19.40 ± 0.1</td>
<td>20.08 ± 0.17</td>
<td>20.53 ± 0.15</td>
<td>20.83 ± 0.05</td>
<td>21.00 ± 0.17</td>
</tr>
<tr>
<td>Palm Kernel Oil</td>
<td>20.5 ± 0.10</td>
<td>20.83 ± 0.05</td>
<td>21.26 ± 0.15</td>
<td>22.6 ± 0.10</td>
<td>23.03 ± 0.20</td>
</tr>
</tbody>
</table>

Table-2: Antibacterial Activity Test of Fatty Acid Salts to Staphylococcus epidermidis

<table>
<thead>
<tr>
<th>Fatty Acid Salts</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Palm Oil</td>
<td>8.93 ± 0.15</td>
<td>-</td>
<td>9.1 ± 0.10</td>
<td>-</td>
<td>9.6 ± 0.2</td>
</tr>
<tr>
<td>RPO with PKO 20%</td>
<td>8.93 ± 0.15</td>
<td>-</td>
<td>9.6 ± 0.2</td>
<td>10.03 ± 0.11</td>
<td>10.23 ± 0.05</td>
</tr>
<tr>
<td>RPO with PKO 50%</td>
<td>18.06 ± 0.15</td>
<td>18.28 ± 0.23</td>
<td>19.66 ± 0.15</td>
<td>20.4 ± 0.26</td>
<td>20.76 ± 0.05</td>
</tr>
<tr>
<td>RPO with PKO 80%</td>
<td>18.7 ± 0.26</td>
<td>20.03 ± 0.30</td>
<td>20.86 ± 0.25</td>
<td>21.43 ± 0.05</td>
<td>21.73 ± 0.11</td>
</tr>
<tr>
<td>Palm Kernel Oil</td>
<td>20.56 ± 0.05</td>
<td>21.23 ± 0.15</td>
<td>21.56 ± 0.15</td>
<td>21.83 ± 0.05</td>
<td>22.6 ± 0.10</td>
</tr>
</tbody>
</table>
The antibacterial test against *Propionibacterium acnes* and *Staphylococcus epidermidis* in RPO fatty acid salts showed no inhibited zone diameter. Meanwhile, the analysis of fatty acid salts RPO: PKO (80:20) showed that the 10-50% concentration was inhibited zone diameter by 20-22 mm, followed by the more significant PKO ratio in the mixture. Fatty acid salts RPO: PKO (50:50) in 10% concentration has shown good antibacterial activity with an inhibited diameter by 18 mm.15 *Propionibacterium acnes* and *Staphylococcus epidermidis* are positive gram bacteria that usually contributed to causing acne.16 Medium-chain fatty acids, such as lauric acid (C12:0), are the most active in fighting positive gram microorganisms.17,18 These will affect the amount of inhibited diameter in fatty acid salts with PKO in the mixture.

**Physical Evaluation of Liquid Soap**

In this research, RPO and PKO with 50:50 combinations were chosen to be formulated as liquid soap preparation with 30% concentration. This combination has a balanced composition of saturated fatty acid and unsaturated fatty acid rather than other combinations. It also contains a high amount of squalene, vitamin E, total carotene, and proper to inhibit bacteria growth criteria (around 20 mm). Hence, this combination was assessed to have appropriate nutrition with optimal antibacterial inhibitor, among other fatty acid salts.19 The physical evaluation of the liquid soap formula was presented in Table-3.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Organoleptic</th>
<th>pH</th>
<th>Specific Weight (g/cm³)</th>
<th>Free Alkaline (%)</th>
<th>Total Plate Count</th>
<th>Foam Height (cm)</th>
<th>Viscosity (Cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Orange, Specific Odor</td>
<td>9.4</td>
<td>1.014</td>
<td>0.071</td>
<td>1x10¹</td>
<td>5</td>
<td>184</td>
</tr>
<tr>
<td>F2</td>
<td>Orange, Specific Odor</td>
<td>9.4</td>
<td>1.029</td>
<td>0.064</td>
<td>1x10¹</td>
<td>6.5</td>
<td>370</td>
</tr>
<tr>
<td>F3</td>
<td>Orange, Specific Odor</td>
<td>9.5</td>
<td>1.046</td>
<td>0.071</td>
<td>1x10¹</td>
<td>8</td>
<td>1,220</td>
</tr>
</tbody>
</table>

Note: (F1) formula with carbomer 1%, (F2) formula with carbomer 2%, (F3) formula with carbomer 3%

The results show that the organoleptic observation from the liquid soap formulation shows that the three formula has a similar color and similar odor, but the liquid soap formula has a different thickness. The liquid soap has a transparent orange color and a specific perfume odor. All of the formulations visually show no separation. All of the liquid soap formulations has pH was 9.4, 9.4, and 9.5, respectively. Based on the Indonesian National Standard,20 the required pH ranged in around 8-11. The test of the pH number of the three formula is still in the specified range. An accurate weight is a ratio of the substantial weight to the same water volume measured on the air with the same temperature. The test result shows that the liquid soap has specific gravity from the first to the third has complied with the requirement of Indonesian National Standard criteria (1.01-1.10). The specific weight of the three formula was 1.014, 1.029 and 1.046 respectively.20 This research uses potassium as the free alkaline while the production of liquid soap preparation used KOH. The residue of alkaline in soap should not exceed 0.1% as alkaline is substantial and could cause skin irritation.20 Simultaneously, lack of free alkaline may cause the exceed of free fatty acid, as KOH did not saponify the fatty acid. The high amount of free alkaline (above 0.22%) is usually also shown by the alkaline pH above 11. The high amount of free alkaline (above 0.22%) is usually also shown by the alkaline pH above 11. The amount of total alkaline residue in the three liquid soap formula was 0.071, 0.064, and 0.071%, respectively.22 The TPC test result shows a negative score (<25 bacteria colony) in each formula. This score is smaller (<) than one multiply by the lowest dilution factor that is 1x10¹. According to the Indonesian National Standard, the maximal TPC number of a qualified soap is 1x10³. It means that the soap produced from this research is qualified as liquid soap according to the Indonesian National Standard.20,23 Foam is one of some parameters in soap formulation stability. The good soap had more foam and more stable foam rather than less foam or unstable foam.24 The foam weight test result from the three formulas is 5 cm, 6.5 cm, and 8 cm.22 Viscosity is one of the variables in consumer acceptance of soap products. The viscosity test of the liquid soap formula was performed by testing the ease
of soap flow from the packaging. The test result shows there is a rise in the used liquid carbomer concentration. ²⁵

**Stability Test**

Stability tests of all liquid soap formulas were prepared by using the freeze-thaw cycling test. The results of liquid soap stability were presented in Table-4.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Cycling test Cycle (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>F1</td>
<td>x</td>
</tr>
<tr>
<td>F2</td>
<td>x</td>
</tr>
<tr>
<td>F3</td>
<td>x</td>
</tr>
</tbody>
</table>

Note: (F1) formula with carbomer 1%, (F2) formula with carbomer 2%, (F3) formula with carbomer 3%, (x) 4°C, (y) 25°C, (z) 40°C

The parts assessed are color-changing, shape, odor, and the viscosity of preparation at the end of the stability test cycle. The excellent result of stability observation was shown through the no color changing, no odor, and no visual separation of the preparation. These results indicate that from the appearance side, the liquid soap preparation with fatty acid salts of RPO: PKO (50:50) is stable, along with the stability test using the cycling test method.²⁶

**Skin Irritation Test**

The irritation test on the volunteers' skin was performed by applying the cosmetic twice to three times a day on the bottom side of the arm for 24 hours. The irritation test result was presented in Table-5.

<table>
<thead>
<tr>
<th>Irritation Symptom</th>
<th>Volunteer Numbers (Person)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itch</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Redness</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coarse skin</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (-) no accident, (+) itch, (++) redness, (+++) coarse skin

The result shows no side effects such as itch, redness, and skin coarse caused by liquid soap formulation while applied on the skin. The irritation test was performed by using the liquid soap preparation on the backside of the ear and was left for 24 hours.²⁷ Irritation can be divided into two categories, such as primary irritation that will be seen directly after liquid soap preparation applied or after in contact with the skin, and secondary irritation which is the reaction that will be seen after some hours after the liquid soap preparation used or in contact with the skin.²⁸

**CONCLUSION**

Fatty acid salts produced from RPO and PKO combination have a more varied characteristic and antibacterial activity. These fatty acid salts can be formulated into face soap cleansers combined with carbomer as a thickener and qualified as liquid soap with Indonesia National Standard No.06-4085-1996.

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