Physical characteristic and antioxidant activities of liquid bath soap with substitution of β-carotene crude extract from *Gracilaria* sp.

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Abstract. Synthetic antioxidant BHT is widely used in bath soap products as an antioxidant and prevents rancidity in bath soaps, but BHT is harmful to the skin if the addition is excessive. So we need natural antioxidants that can replace the role of BHT as an antioxidant. The purpose of this study was to determine the effect of substitution of BHT with β-carotene *Gracilaria* sp. on the physical properties of liquid bath soap and determine the best concentration of BHT substitution with β-carotene *Gracilaria* sp. added to liquid bath soap formulations. This study used a completely randomized design (CRD) consisting of 3 substitution treatments for BHT with β-carotene, namely (1g BHT : 0g β-carotene), (0,5g BHT : 0,5g β-carotene), and (0g BHT : 1g β-carotene) in 100 ml of liquid soap, with 6 repetitions each. Parameters observed were pH, foam stability, viscosity, hedonic, physical stability, and antioxidant activity. The results of this study the substitution of β-carotene *Gracilaria* sp. the liquid bath soap had a significant effect (p<0.05) on the pH parameter and no significant effect (p<0.05) on the foam stability test and viscosity test parameters. Based on the hedonic test value, soap with the addition of 0,5g BHT: 0,5g β-carotene has the highest test value to produce the best quality soap.

1. Introduction

People with degenerative diseases in recent years have increased in number caused by excessive free radical activity in the body. UV radiation can form free radicals that have a negative impact on the body if exposed for too long causing redness of the skin so that the skin feels burned and results in skin cancer [1]. Antioxidant compounds can inhibit the formation of free radicals. This compound has been widely added to the cosmetic industry to prevent the formation of free radicals on the skin, one of which is the antioxidant BHT (buthylated hydroxytoluene). Bath soap is a skin health product that BHT often adds in its formulation. However, the use of BHT as an excessive synthetic antioxidant is considered less safe for the skin because it can cause irritation of the epidermis [2]. So, that natural antioxidant compounds are more recommended for use because they have a better level of safety such as β-Carotene compounds. β-carotene compounds...
can be found in plants such as seaweed, one of which is *Gracilaria* sp. β-Carotene in *Gracilaria* sp. It has potential as an antioxidant by inhibiting damage caused by ROS (oxygen-containing free radicals). Based on the description above, it is necessary to do research to find out the potential utilization of coarse extracts β-Carotene from *Gracilaria* sp. In replacing the role of BHT as an antioxidant liquid bath soap and its effect on the physical characteristics of liquid bath soap.

2. Material and Method

2.1 Materials

The tools used in this study were 1000 mL Erlenmeyer, 1000 mL measuring cup, aluminum foil, blender, petri dish, rotary evaporator, separating funnel, filter paper, measuring pipette, volume pipette, dropper pipette, stirring rod, analytical balance, centrifuge, refrigerator, sieve, plastic, 600 mL beaker glass, spectrophotometry, test tube, cuvette, test tube rack, plastic wrap, cotton swab, pH meter, pycnometer The research materials used were *Gracilaria* sp. seaweed, ethanol, ethyl acetate, 30 ml castor oil, 45 ml olive oil, 30 ml coconut oil, 15.45 g KOH, 9 g HMPC, 6 g stearic acid, 56.25 g glycerin, BHT 2 g, distilled water, methanol 96%.

2.2 Extraction of β-carotene *Gracilaria* sp.

Crude extract of β-Carotene was obtained from extraction using the method of Yulianti et al [4] modified. Seaweed *Gracilaria* sp. 200 g powder was dissolved in 1 liter of cold hexane and petroleum solvent mixture (50:50) homogenized with an orbital shaker (15 minutes, 250 rpm) and allowed to stand for 15 minutes. The filtrate is filtered using a vacuum pump. and stored in the refrigerator for 40 hours at 4 oC. then in cold centrifugation at a speed of 4600 rpm for 40 minutes at a temperature of 5°C. The filtrate was then evaporated using a rotary evaporator to remove solvent at a roto speed of 50 rpm with a water bath temperature of 50°C. The extract was then allowed to stand until the extract was in the form of a paste (thick) at room temperature of ± 25°C.

2.3 Measurement of β-carotene content.

10 mg of β-carotene extract of *Gracilaria* sp., then dissolved in a 10 mL volumetric flask with ether to the mark, then shaken and the absorption measured at a wavelength of 450 nm using spectrophotometry UV-VIS (replication was performed 3 times).

2.4 Soap Making Process.

Castor oil is mixed with olive oil and VCO, stirred slowly until homogeneous. KOH solution with a concentration of 10% is added little by little into the oil mixture at a temperature of 60-70°C until a paste is formed. Then, stearic acid, which had previously been melted, was added and stirred until homogeneous. BHT and HPMC, which had been developed in hot distilled water, were added to the mixture. Then, glycerin and extract were added and stirred until homogeneous. Next, distilled water was added up to 100 ml and then stirred until homogeneous and put into a container.

2.5 Physical Properties of Liquid Bath Soap

Physical properties test of liquid bath soap includes hedonic test, foam stability test, viscosity test, pH test, physical stability test of soap.

a. Hedonic Test

Hedonic test is a way of testing using human senses as the main tool to measure the level of liking for the product. Tests in measuring the level of preference for the product are carried out based on a scale of 1 (one) as the lowest value and 9 (nine) as the highest value using an assessment sheet.
Samples are presented simultaneously to the panelists and assessed based on the level of preference using a score of 1 – 9 for each parameter. Parameters of this hedonic test include color, texture, and smell.

b. Foam Stability Test
The sample was weighed as much as 1 g, put into a test tube, then added up to 10 ml of distilled water, shaken by inverting the test tube, and immediately measuring the height of the foam produced. Then, the tube was allowed to stand for 5 minutes, then the height of the foam produced was measured again after 5 minutes.

\[
\text{Foam Test: } \frac{\text{Final foam height}}{\text{Initial foam height}} \times 100 \%
\]

c. Viscosity Test
Viscosity was measured using an Ostwald viscometer by entering water in tube A into the Ostwaldm viscometer, water was used as a comparison. Then the water is sucked into tube B right up to the a limit then released and the speed of water flowing through the capillary tube required by the water to pass through the “A” and “B” limits is measured using a stop watch. Sample measurements were carried out in the same way. Furthermore, the viscosity of the sample can be measured using the formula Gel Strength

\[
\eta = \eta_0 \frac{t \cdot \rho}{to \cdot \rho_0}
\]

d. pH
pH testing is done using a pH meter. The pH meter is calibrated with a buffer solution every time a measurement is made. The electrode, which has been cleaned, is immersed in the sample to be examined. The pH value on the pH meter scale is recorded.

e. Physical Stability Test
This test uses the mechanical test method, the liquid soap sample is centrifuged with a rotation speed of 10,000 rpm for 30 minutes, because the results are equivalent to the effect of gravity for 1 year. Then visually observed.

f. Antioxidant Activity Test
Antioxidant activity test using the DPPH method which begins with making a standard solution of DPPH by weighing 0.01 g, then the results of the scales are homogenized using 100 ml of methanol solvent. Next is the manufacture of 40 ppm (0.004%) DPPH solution by taking a 100 ppm DPPH solution with a pipette of 20 ml. The solution was put into a 50 ml volumetric flask, then diluted with methanol and homogenized. The first measurement was carried out on the blank solution by making 3 ml of 0.004% DPPH solution and then adding 3 ml of methanol. The solution was homogenized and incubated for 25 minutes at 37°C. Furthermore, the absorbance of the blank was measured using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm. The next measurement is carried out on liquid bath soap. Liquid bath soap is pipetted into a test tube as much as 3 ml and then 3 ml of 0.004% DPPH solution is added. Then the solution was homogenized, then incubated for 25 minutes at 37°C. Then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The next step is to calculate the percentage of inhibition and the IC50 value. The percentage of inhibition was calculated using the formula

\[
\text{% Inhibition} = \left[1 - \left(\frac{A \text{ sample}}{A \text{ Control}}\right)\right] \times 100\%
\]
3. Results and Discussion

3.1. Analysis of β-carotene extract *Gracilaria* sp.

**Table 1.** Measurement Results Levels of crude extract of β-Carotene *Gracilaria* sp.

| Sample     | Weight (gram) | Average Absorbance (450 nm) | Content (mg/g) |
|------------|---------------|----------------------------|---------------|
| *Gracilaria* sp. | 200           | 0.4796                     | 0.0177        |

Crude extract of β-Carotene obtained from the extraction of seaweed *Gracilaria* sp. dry as much as 200 g of 0.0177 mg/g. Levels of β-Carotene *Gracilaria* sp. higher than the results of β-Carotene levels of red seaweed *Acanthopora muscoides* in which is 0.01647 mg/g. The difference in levels is caused by several factors such as differences in seaweed species, season, habitat, chemical composition, differences in harvest time or differences in seaweed locations.

3.2. Foam Stability

The stability test of liquid soap foam on BHT substitution treatment for β-Carotene (0%, 50%, 100%) was carried out after the first, seventh, and fourteenth day of storage. Based on the graph in Figure 1, it can be seen that the stability value of liquid soap foam with BHT substitution for β-Carotene (0%, 50%, 100%) increased the foam stability value after the 7 and 14 days storage. Substitution of BHT with β-Carotene in liquid bath soap with different concentrations (0%, 50%, and 100%) has a high foam value of about 78–88 mm and foam stability of 78.6–88.6%. These results have met the requirements for the height of the liquid soap foam, which is 13-220 mm and good foam stability has a value above 70%. Foam stability is influenced by surfactant concentration, temperature, type of solvent and oil in the soap formulation [3].

![Figure 1](image_url). The stability of liquid soap foam with substitution of β-carotene 0%, 50%, 100% (P1,P2,P3) after 14 days of storage.
3.3. pH
The pH test of liquid bath soap on BHT substitution treatment for β-carotene (0%, 50%, 100%) was carried out after the first, seventh, and fourteenth day of storage. The results of pH testing on liquid bath soap with BHT substitution for -Carotene (0%, 50%, 100%) can be seen in Figure 2 shows that the pH value of liquid bath soap with BHT substitution for -Carotene (0%, 50%, 100%) increased the pH value after the 7 and 14 days storage. Substitution of BHT with β-carotene in liquid bath soap with different concentrations (0%, 50%, and 100%) had pH values ranging from 8.3 to 9.6 after 14 days of storage. The results of the pH of the liquid bath soap in this study have met SNI Soap Mandi No. 06-4085-1996 which ranges from 8-11.

![Figure 2. pH test result of liquid soap foam with substitution of β-carotene 0%, 50%, 100% (P1,P2,P3) after 14 days of storage.](image)

3.4. Viscosity test
The viscosity test of liquid bath soap with BHT substitution for β-carotene (0%, 50%, 100%) was carried out after the first, seventh, and fourteenth day of storage. The results of viscosity testing on liquid bath soap with BHT substitution for β-carotene (0%, 50%, 100%) can be seen in Figure 3. It is known that the viscosity value of liquid bath soap with BHT substitution for β-carotene (0%, 50%, 100%) decreased in viscosity value after the 7 and 14 days storage. The decrease in the viscosity of liquid bath soap can be caused by the evaporation of several components that make up liquid bath soap [4]. Viscosity is influenced by thickening agent, selected surfactant, proportion of dispersed phase and particle size [5].
3.5. Hedonic test
Hedonic testing of liquid bath soap with BHT substitution for β-carotene (0%, 50%, 100%) was carried out after the first, 7 and 14 days storage by covering 3 parameters color, texture and odor. The results of hedonic testing on liquid bath soap with BHT substitution for β-carotene (0%, 50%, 100%) can be seen in Figures 4, 5 and 6. Liquid bath with BHT substitution for β-carotene (0%, 50%, 100%) with color, texture, and odor parameters showed different improvements in each treatment.

Figure 3. pH test result of liquid soap foam with substitution of β-carotene 0%, 50%, 100% (P1, P2, P3) after 14 days of storage.

Figure 4. Hedonic results of liquid soap foam with substitution of β-carotene 0%, 50%, 100% (P1, P2, P3) with the parameters of color, texture, and odor after 1 day of storage.
The results of the 7 days hedonic test value in Figure 5. can be seen that liquid bath soap with BHT substitution for β-carotene (0%, 50%, 100%) with texture parameters showed an increase but in color and odor parameters decreased.

![Figure 5](image1.png)

**Figure 5.** Hedonic results of liquid soap foam with substitution of β-carotene 0%, 50%, 100% (P1,P2,P3) with the parameters of color, texture, and odor after 7 days of storage.

The results of the hedonic test on 14 days of storage in Figure 6. It can be seen that liquid bath soap with BHT substitution for β-carotene (0%, 50%, 100%) with texture and odor parameters showed an increase but the color parameter decreased.

![Figure 6](image2.png)

**Figure 6.** Hedonic results of liquid soap foam with substitution of β-carotene 0%, 50%, 100% (P1,P2,P3) with the parameters of color, texture, and odor after 7 days of storage.
Substitution of BHT with β-carotene in liquid bath soap with different concentrations (0%, 50%, and 100%) produces different colors. This is due to the addition of β-carotene extract which contains yellow to red-orange organic pigments [6]. The difference in the color results of β-carotene can be influenced by several factors such as the chemical structure of β-carotene on the type or species, the concentration of the sample to be tested, the temperature in the extraction process, the effect of oxygen and the experimental technique. Substitution of BHT with β-carotene in liquid bath soap with different concentrations (0%, 50%, and 100%) did not affect the panelists’ preference for texture parameters. The liquid bath soap in this study used the same oil and HPMC formulations between treatments so that the texture of each treatment did not differ. Substitution of BHT with β-carotene in liquid bath soap with different concentrations (0%, 50%, and 100%) affected the panelists’ preference for odor parameters. The liquid bath soap in this study was not added with fragrance so that the smell produced was a distinctive aroma from the β-carotene extract of *Gracilaria* sp., states that the odor test of VCO liquid bath soap containing carrot extract shows that the liquid bath soap has a distinctive odor, which is a combination of the aroma of VCO and a slight carrot aroma which is rich in β-carotene [7].

3.6. Physical Stability

Physical stability test performed using a mechanical method, in which a sample of liquid bath soap disentifugasi (6000 rpm, 18 minutes) and then observed visually. These results are equivalent to the effect of gravity for 1 year.

| Table 2. Physical Stability Test Results Liquid Bath Soap |
|----------------------------------------------------------|
| Treatment | Texture | Color | Formed Phases |
|------------|---------|-------|---------------|
| P1         | Thick   | Clear | Formed 2 phases |
| P2         | Thick   | Clear | Formed 2 phases |
| P3         | Thick   | Clear | Formed 2 phases |

Based on the results of Table 2, it can be seen that the overall physical stability of liquid bath soap with BHT substitution for β-carotene has a clear color, thick texture and is formed in 2 phases. Substitution of BHT with β-carotene (0%, 50%, and 100%) in liquid bath soap, after being centrifuged the color, smell, texture changed and separated into 2 phases. This phase separation occurs because the liquid bath soap is not homogeneous. This homogeneity is influenced by the ingredients contained in the liquid bath soap. The formulation of liquid bath soap substitutes BHT with β-carotene cannot blend perfectly with other soap ingredients because there is no addition of surfactant. Surfactants function as compounds that can lower the voltage between two liquid phases of different polarity [8].

3.7. Antioxidant Activity

The antioxidant activity of liquid bath soap with crude extract of β-carotene was analyzed using the DPPH method. Measurement of antioxidant activity by UV-VIS spectrophotometry at a wavelength of 517 nm maximum DPPH [9]. Based on the calculation results of the antioxidant activity test in Table 3, it can be seen that the percentage of inhibition in all treatments, namely treatment P1 (0%) was 17.34%, treatment P2 (50%) was 18.87%, and treatment P3 (100%) was 17.85%.
Table 3. Calculation Result of Percent Inhibition Measurement (%) Antioxidant Activity with DPPH method

| Treatment | Concentration | Absorbance | Percent Inhibition (%) |
|-----------|---------------|------------|------------------------|
| Control   | 100 ppm       | 0.196      | -                      |
| P1        | 100 ppm       | 0.162      | 17.34%                 |
| P2        | 100 ppm       | 0.159      | 18.87%                 |
| P3        | 100 ppm       | 0.161      | 17.85%                 |

Substitution of BHT with β-carotene in liquid bath soap with different concentrations (0%, 50%, and 100%) had almost the same antioxidant activity between treatments and BHT. This is because the BHT and extract β-carotene has the same function as an antioxidant in the soap so that it can be concluded that BHT can be replaced by β-carotene as an antioxidant in the soap.

4. Conclusions
BHT substitution with β-carotene extract of Gracilaria sp. on liquid bath soap had a significant effect on physical characteristics on pH parameters and odor parameters on hedonic tests, but had no significant effect on physical characteristics on parameters of viscosity, foam stability, physical stability, hedonic test (color and texture) and antioxidant activity of liquid bath soap. Substitution of BHT and β-carotene extract with a comparative concentration (50%) is the optimal concentration in the formulation of liquid bath soap.

5. References
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