The storage time on the characteristic of liquid dishwashing soap from nyamplung seed oil (*Calophyllum inophyllum* L) and its antibacterial activity

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The storage time on the characteristic of liquid dishwashing soap from nyamplung seed oil (*Calophyllum inophyllum* L) and its antibacterial activity

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Abstract. The production of liquid dishwashing soap from nyamplung seed oil had been studied to obtain the optimum soap formula and determine its storage time. Liquid dishwashing soap is produced through the saponification process using KOH with variation of nyamplung seed oil content. The optimum soap formula was used to determine the storage time that was carried out in 12 weeks. The antibacterial activity of this product was determined through antibacterial activity tests using *S. aureus* and *E. coli* bacteria. Soap characteristics which include pH, specific gravity, free fatty acids, unsaponified fat, total fatty acids and pelican oil are determined every week. The results showed that the optimum formulation was liquid dishwashing soap with oil content 40%. The optimum formulation characteristics were 10.166 for pH, 1.040 for specific gravity, 0.54% for free fatty acids, 4.85% for unsaponified fat, 33.47% for total fatty acids, and negative pelican oil. The result of time series test showed that the storage time of liquid dishwashing soap from nyamplung seed oil is 49 weeks, while antibacterial test result showed the formation of inhibitory zones against *S. aureus* bacteria before storage by 40.42 mm and after storage by 15.57 mm, while against E. coli bacteria had a 10.60 mm inhibition zone before storage and 6.88 mm after storage.

Keywords : antibacterial, dishwashing, nyamplung, saponification, soap

1. Introduction

One of the innovations that has been developed is the use of nyamplung (*C. inophyllum* L) seed oil as basic ingredients of soap, with addition of active substances such as antibacterial and fragrance. The results showed that the best characteristic soap according to SNI 06-4085-1996 was soap with 0.2% antibacterial content and 2.5% perfume. The best soap characteristic showed total fatty acid 67.49%, free fatty acid 1.01%, neutral fat 7.24%, weight of type 1.05 g/mL, pH of 9.85 and foam stability 76.69%. The results of the antibacterial activity test of the best formula soap showed the inhibitory zone of 14.92 mm.

The production of dish soap in Indonesia still uses edible oil as a basic ingredient. Various studies using non-food oils as basic ingredients for soap making have been widely carried out. Widyaningsih *et al.* [1] uses nyamplung seed oil as the basic material for the liquid dishwashing antibacterial soap. Nyamplung seeds are used because they contain 50-70% of oil. In this study nyamplung seed oil was used in the production of liquid dishwashing soap. Liquid soap is preferred than solid soap because it is

easy to store and use [1]. Soap is formed through a saponification reaction. Saponification is a reaction between oil and alkali that produces soap. The alkali that was used are NaOH and KOH. The use of KOH is known to produce softer and liquid form of soap [2].

Several studies on soap were only carried out to the production process while the storage time has never been reported. Determination of storage time needs to be studied expected as a basic information in producing soap on a small industry. In addition, soap is used because of its nature as a cleanser from dirt and bacteria. The bacteria that are generally in the environment are *S. aureus* and *E. coli* [3]. In order to know its antibacterial activity, it is necessary to test antibacterial activity of the soap against the *S. aureus* and *E. coli* bacteria. The purpose of this study was to determine the optimum content of nyamplung seed oil which produced the best characteristics corresponding of Indonesian National standard and found out the storage time and antibacterial activity.

2. Experimental

2.1. Materials

The research used nyamplung seeds obtained from Gunung Selok Kroya, KOH, S. aureus bacteria, *E. coli* bacteria, NA media (Nutrient Agar), NB media (Nutrient Broth), and tetracycline. The equipment used in this study was a hotplate stirrer, rotary evaporator, pH meter, UV-Vis spectrometer, a set of reflux devices, a set of distillation devices, a set of sterilizers, petri dishes, ose needles, drugal sky, and micropipette.

2.2. Preparation of liquid dishwashing soap from nyamplung seed oil

A total of 20 g of nyamplung seed oil were heated until the temperature reached 70^{o}C. 10.6 grams of 30% KOH solution (b/v) was added and heated for 60 minutes. Additive such as texapon, glycerine, perfume, and dye were added while stirring process. The treatment is repeated for different formulations. Liquid dishwashing soap formulations can be seen in Table 1.

Table 1. Formulation of liquid dishwashing soap from nyamplung seed oil.

Ingredients	Formulation (% (b/b))					
	I	II	III	IV	V	VI
Nyamplung seed oil	20	25	30	35	40	45
KOH 30%	10.6	13.3	15.9	18.6	21.2	23.9
Texapon	5	5	5	5	5	5
Glycerine	5	5	5	5	5	5
Perfume	0.5	0.5	0.5	0.5	0.5	0.5
Dye	0.2	0.2	0.2	0.2	0.2	0.2
Water	58.7	51	43.3	35.7	28.1	20.4

2.3. Characterization of soap products

Liquid dishwashing products are characterized in accordance with SNI 06-06-2048-1990, including: pH, specific gravity, free fatty acids, unsaponified fat, total fatty acids, and pelican oil.

2.4. Data analysis

The experimental design used in the determination of liquid dishwashing soap with the best characteristics is a non-factorial completely randomized design. The independent variable is the nyamplung seed oil content, while the dependent variable is the characteristic of soap. The data were

analysed using ANOVA test (Analysis of Variants). If the results show a significant difference, then proceed with the DMRT test (Duncan's Multiple Range Test) with a 95% confidence level ($\alpha = 0.05$).

2.5. Determination of storage time

Liquid dishwashing soap was inserted into 13 containers, each containing 25 grams of soap and characterized. Characterization is carried out every week from zero weeks (T0) until 12th weeks (T12).

2.6. Antibacterial activity test of liquid dishwashing soap

The antibacterial activity test of liquid dishwashing soap was performed by pouring 15 mL of Nutrient Agar (NA) medium at ± 40°C into sterile petri dish then left at room temperature until the medium to solidify. A number of bacterial cultures in Nutrient Broth (NB) liquid medium are taken and dispersed in Nutrient Agar (NA) medium. The volume of bacteria taken based on the absorbance results at 600 nm. If the absorbance value is less or equal to 0.5 then is taken 100 µL bacterial culture, whereas if 0,6-1,0 is taken 50 µL. The suspension of the test bacteria on Nutrient Agar (NA) medium was streaked on the spread plate by using drugalsky, then allowed to dry for 15 minutes at room temperature. After drying, a paper disc with a diameter of ± 6 mm is placed over the NA medium. Samples and controls were taken as much as 10 µL and dripped onto paper disc then incubated for 24 hours at 37°C. Then it was measured the inhibitory diameter formed around the disc paper using calipers.

3. Result and Discussion

3.1. Characteristics and formulations of liquid dishwashing soap

Characteristics of liquid dishwashing soap are pH, specific gravity, free fatty acids, unsaponified fat, total fatty acids, and pelican oil. The results obtained were compared with SNI 06-06-2048-1990. Determination of the best liquid dishwashing formulation is based on the physicochemical analysis of each formulated liquid dishwashing soap. The method used is the effectiveness index [4] with the principle of determining the observation parameters according to priority. The weight is determined, determining the lowest value, highest value and treatment value, so that the value of effectiveness can be calculated. The results showed that the best formulation is the 40% for nyamplung seed oil content with an effectiveness index value of 0.6579. The characteristics of liquid dishwashing soap from nyamplung seed oil from the formulation can be seen in Table 2.

Table 2. Characteristic of liquid dishwashing soap from nyamplung seed oil.

Characteristic	Nyamplung seed oil content (%)						SNI
	20	25	30	35	40	45	
pH	10.346	10.285	10.269	10.228	10.166	10.196	8-11
Specific gravity (g/mL)	1.024	1.029	1.032	1.035	1.040	1.045	1.010-1.100
Free fatty acid (%)	0.21	0.33	0.36	0.48	0.54	0.57	< 2.5
Unsaponified fat (%)	3.70	3.93	5.04	5.11	4.85	6.43	< 2.5
Total fatty acid (%)	11.95	17.34	20.31	26.88	33.47	36.37	> 15
Pelican oil	Negative	Negative	Negative	Negative	Negative	Negative	Negative

3.2. Characteristics of liquid dishwashing soap in various storage times

3.2.1. pH. The pH of liquid dishwashing soap from zero weeks (T0) until the 12th weeks (T12) can be seen in Fig. 1. Fig. 1. shows that from zero week (T0) until 12th week (T12) pH has decreased. It is caused during the storage; weak acids are formed by microbial activity. Microbes can come from raw materials during packaging, producing, or storing. Data analysis using Pearson correlation test shows

that there is a relationship between pH and storage time. The relationship between pH and storage time is at a correlation level of -0.980, which means that the level of correlation is at a strong correlation level with a negative influence. The longer the storage time, the lower pH value.

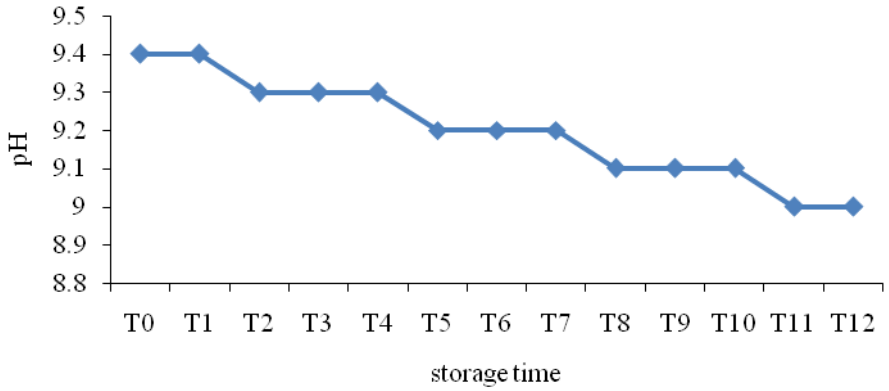

Figure 1. The effect of storage time (week) on pH.

Further data analysis was carried out by extrapolation test using time series method to determine the pH value limit that was not included in SNI (1990) [5]. According to SNI (1990), a good liquid dishwashing soap has a pH value of 8-11. Based on time series method analysis data, the storage time for the liquid dishwashing soap is 44 weeks.

3.2.2. Specific gravity. The results of measuring the specific gravity of dish soap from zero week (T0) until 12th week (T12) can be seen in Fig. 2.

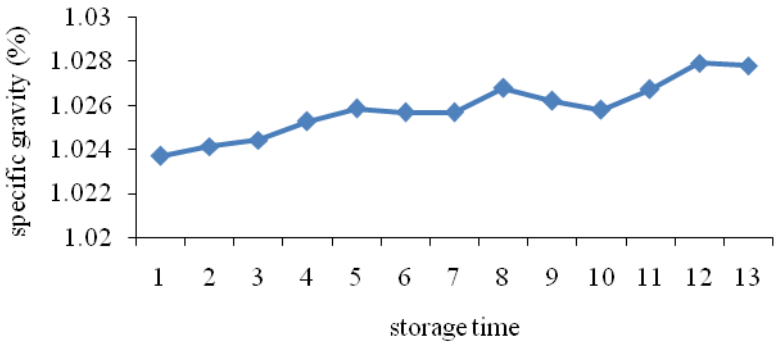

Figure 2. The effect of storage time (week) on specific gravity.

Fig. 2. shows that the specific gravity of liquid dishwashing soap from zero week (T0) until 12th week (T12) is quite stable during storage time. Although there is an increase or decrease but the change is very small, so it does not affect the stability of soap during 12 weeks storage [6]. At the beginning of the process there is still foam trapped in the soap which causes a large distance between the soap molecules and causes the density to tend to be low. The longer the storage time, the more stable the soap will be and the distance between the soap molecules is getting closer, causing the higher specific gravity. Based on data analysis using Pearson correlation test, there is a relationship between specific gravity and storage time. The relationship between the specific gravity and storage time is at the level of correlation of 0.935, which means that the level of correlation is at a strong correlation level with a positive influence, namely the longer the storage time, the higher the specific gravity value. The results of time series data analysis of the specific gravity of liquid dishwashing soap have a storage time limit of 128 weeks.

3.2.3. Free fatty acids. Free fatty acid of liquid dishwashing soap levels from zero week (T0) until 12th week (T12) can be seen in Fig. 3.

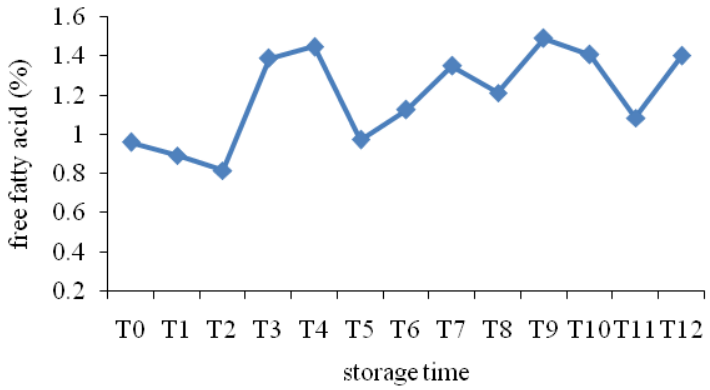

Figure 3. The effect of storage time (week) on free fatty acid

Fig. 3 shows that the levels of free fatty acids from zero week (T0) until 12th week (T12) tend to rise. This increase is due to the less stable of soap during storage time. Oxidation reactions can occur due to the influence of light and oxygen when storing soap in transparent glass bottles. The combination of oxygen and light can accelerate the oxidation process, thereby increasing the amount of free fatty acids [7]. The results of data analysis showed a relationship between free fatty acids and storage time. The relationship between free fatty acids and storage time is at a level of correlation of 0.567, which means that the level of correlation is at a moderate level of correlation with a positive influence, namely the longer the storage time, the higher levels of free fatty acids. The results of time series data analysis, provide that liquid dishwashing soap has a time limit of 49 weeks.

3.2.4. Unsaponified fat. Unsaponified fat of liquid dishwashing soap from zero week (T0) until 12th week (T12) can be seen in Fig. 4.

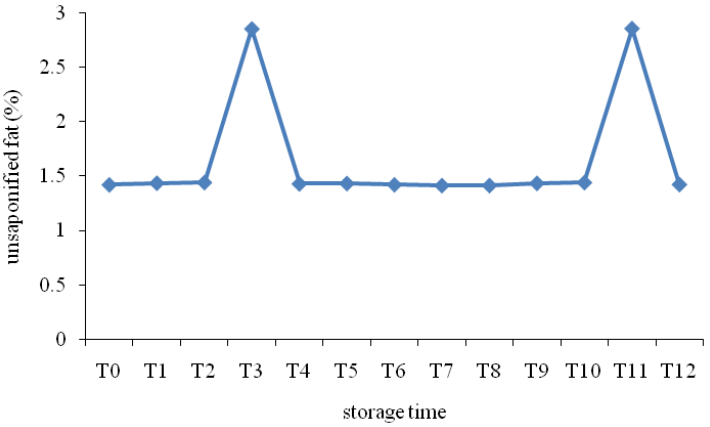

Figure 4. The effect of storage time (week) on unsaponified fat

Fig. 4. shows that the unsaponified fat of liquid dishwashing soap from zero week (T0) until 12th week (T12) is quite stable in the range of 1.5% but there is an increase in certain weeks to around 2.8% and back fell again the following week. This is because the soap is not homogeneous when it is inserted into tubes when storage time test. The results of the data analysis showed that there was no relationship between unsaponified fat and storage time. According to [8], unsaponified fat are long-chain alcohols,

sterols, hydrocarbons and pigments or dyes that are added to the soap. These components tend to be stable during storage.

3.2.5. Total fatty acid. Total fatty acid of liquid dishwashing soap from zero week (T0) until 12th week (T12) can be seen in Fig. 5.

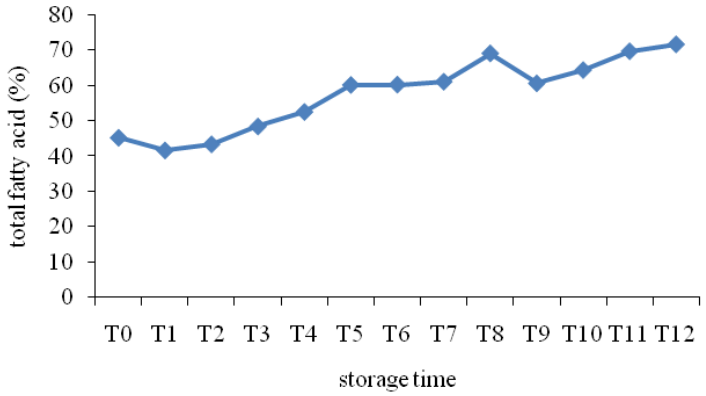

Figure 5. The effect of storage time (week) on total fatty acid

Fig. 5 shows that the levels of total fatty acids from zero week (T0) until 12th week (T12) tend to rise. The results of data analysis showed a relationship between total fatty acids and storage time. The relationship between total fatty acids and storage time is at a level of correlation of 0.941, which means that the level of correlation is at a strong level of correlation with a positive influence. The longer the storage time, the higher levels of total fatty acids. The results of time series data analysis, provide that liquid dishwashing soap has a storage time limit of 24 weeks.

3.2.6. Pelican oil. Pelican oil test showed a negative result which was seen on unclear solution. A good soap has no pelican oil because it will increase the hardness of soap. Mineral oil is metals or mineral which dissolved into oil.

3.3. Determining the storage time of liquid dishwashing soap

The storage time of a product is defined as the time needed to maintain the quality or characteristics of a product during the storage period [9]. Some of the main factors that result in a decreasing quality or damage to a product are mass oxygen, water vapour, light, microorganisms, and toxic chemicals. These factors cause an oxidation reaction. The oxidation reaction causes the levels of free fatty acids in the soap to increase. The characteristics that most determine the quality of soap during storage are free fatty acids so that determining the storage time of liquid dishwashing soap is based on free fatty acid data.

Based on the results of time series analysis method, free fatty acid data from zero weeks to 12 weeks were not stationary, as seen from the t-Statistic value of -3,044855 (t-Statistic <critical value). The data is then stationed against the mean and variance so as to produce the t-Statistic value of -8.026544 (t-Statistic> critical value). From the stationary data, a test model can be selected which is used to extrapolate the value of free fatty acids. Based on the extrapolation, in the 49th week the free fatty acid data exceeded the SNI limit (2.5%) so that the 49th week was the storage time limit for the soap.

3.4. Antibacterial activity of liquid dishwashing soap

Antibacterial activity was carried out on *S. aureus* and *E. coli*. *S. aureus* is one of bacterial that can cause infection on a skin while *E. coli* is a bacterial that can cause diarrhoea, intestines infection, or another digestion disease.

The method is the disc diffusion method using NA (Nutrient Agar) media. Disc diffusion method is carried out using paper disc containing active compounds [10]. Positive control used in this study is tetracycline antibiotics, while for negative controls used distilled water (test sample solvents). The use

of negative controls was carried out with the aim as a comparison that the solvents used as diluents did not affect the test results [11]. The result of antibacterial activity test can be seen on Table 3.

Table 3. Antibacterial activity on *S. aureus* and *E. coli*

Sample	Inhibition diameter (mm)	
	S. aureus	*E. coli*
Distilled water (-)	-	-
Tetracycline (+)	48,375	22,375
Soap T_0	40,425	10,6
Soap T_{12}	15,575	6,8875

The result showed that liquid dishwashing soap from nyamplung oil can inhibit *S. aureus* and *E. coli*. Inhibition zone for *S. aureus* has a higher activity than *E. coli*. Structure of *S. aureus* bacterial is more simple that antibacterial agent can penetrate easily, find the target, and inhibit bacterial growth. While *E. coli* has a more complex of cells that can inhibit antibacterial agent to penetrate [12].

4. Conclusion

The best formulation of liquid dishwashing soap from nyamplung seed oil is a formulation with oil content of 40%, a storage time of 49 weeks, and has antibacterial activity against S. aureus bacteria before storage by 40.42 mm and after storage by 15.57 mm, while against E. coli bacteria had a 10.60 mm inhibition zone before storage and 6.88 mm after storage.

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