

**Formulation and Stability of Microemulsion Preparations From Secang Wood Extract (Caesalpinia Sappan L) and Virgin Coconut Oil (VCO)****Ani Setyopratiwi, Fathru Naban Rahman, Niko Prasetyo**

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**ABSTRACT**

Research has been conducted to make microemulsion preparations from virgin coconut oil (VCO) and secang wood extract. The purpose of this study was to determine the characteristics of secang wood extract with ethanol solvent, determine the Hydrophilic Lipophilic Balance (HLB) value of a mixture of three surfactants that can form the most stable microemulsion preparations, and determine the most optimal variation in the concentration of secang wood extract in secang microemulsion formulations. This study began with the manufacture of secang wood ethanol extract from secang wood powder and 96% ethanol, then tested for flavonoid levels. Furthermore, VCO microemulsions in a mixture of water with secang extract are made with variations in HLB values, namely 12, 13, 14, and 15. Microemulsions with HLB variations were tested for stability through organoleptical observations and turbidity values by storage treatment at room temperature, heating, and centrifugation. Microemulsions were also tested for free fatty acid (FFA) levels. After that, the preparation of microemulsions with the most stable HLB is carried out with variations in the concentration of secang wood extract in the formulation. Microemulsions with extract variations were tested for stability through organoleptical observation and turbidity values after 24 hours, heating, and centrifugation. Microemulsions with optimal concentrations of secang wood extract were tested for antioxidant activity. The secang extract produced from the extraction was reddish-brown in color and had total flavonoids equivalent to 230.6 mg QE/g samples. The most stable microemulsion from this study is a microemulsion with an HLB value of 15 with a ratio of water:extract:VCO:surfactant which is 65:2,5:2,5:30. The microemulsion features transparent red, 1 phase, and smells like Tween 80 with turbidity value below 1% during storage, heating, and centrifugation with microemulsion FFA value of 0.53%. Based on variations in the concentration of secang wood extract, the higher the concentration of the extract, the more unstable the microemulsion produced. The 3% secang extract microemulsion is the most optimal microemulsion with antioxidant activity (IC<sub>50</sub>) of 1.4573±0.2694 mg/mL.

**Keywords:** microemulsion, secang, surfactant, virgin coconut oil.**INTRODUCTION**

According to BAPPENAS (2016) Indonesia has a high diversity of flora, which ranges from 30,000 to 40,000 species of seed plants. The high diversity of plant species has great potential for the discovery of the bioactive compounds they contain. Several studies have succeeded in revealing the antioxidant potential of several Indonesian plants (Rohman et al., 2007). Antioxidants are substances needed by the body to neutralize free radicals because they have the ability to donate electrons to stabilize free radicals (Vaya and Aviram, 2001). Natural antioxidants



are found in various types of plants in the form of carotenoids, vitamins, flavonoids, and phenols (Mandal et al., 2009).

Secang (*Caesalpinia sappan* L.) is a shrub plant member of the legume tribe that is used bark and wood as a spice trade commodity. The plant is naturally spread throughout Asia, including China, Japan, and Thailand. This plant is also cultivated in various other regions of the world, including Africa, Europe, and North and South America (Ngamwonglumlert et al., 2020).

In various regions of Indonesia, secang wood is used as a medicine for tuberculosis (TB), diarrhea, and dysentery. Secang wood is also efficacious to activate blood flow, dissolve blood clots, reduce swelling, and relieve pain (Dalimartha, 2009). According to Nirmal et al. (2015), secang wood has various phenolic compounds that are beneficial including xanthenes, coumarins, chalcones, flavones, homoisoflavonoids, and brazilin. Brazilin is the main active compound of secang which has antibacterial, anti-inflammatory, and antioxidant activity. The presence of flavonoids and other phenolic compounds in secang wood indicates that secang has potential as an antioxidant (Hu et al., 2008).

Virgin coconut oil (VCO) is a vegetable oil obtained from the flesh of fresh and ripe coconut fruit (*Cocos nucifera* L.), a tropical plant belonging to the family Arecaceae. VCO is a colorless oil with fresh coconut aroma, and has been widely used for cooking, bread, confectionery, baby food, and cosmetics (Marina et al., 2009). VCO, one of the products derived from coconut, has become part of people's daily lives because of its many medicinal properties. These include anti-inflammatory, analgesic, antipyretic, antioxidant, anti-stress, and antimicrobial properties (Dumancas et al., 2016).

In cosmetics, VCO is used as an ingredient to enhance beauty, promote hair growth, and repair and moisturize the skin due to the health benefits of the medium-chain fatty acids contained in VCO. Medium-chain fatty acids such as lauric, myristic, palmitic, capric, stearic, oleic, and linoleic acids are easily digested. VCO contains 90% saturated fatty acids and 10% unsaturated fatty acids. Saturated fatty acids in coconut oil are almost dominated by lauric acid (DebMandal and Mandal, 2011).

Secang wood extract has many benefits, one of which is as an active antioxidant compound, but the polyphenol content in secang wood can experience instability that decreases the antioxidant activity of secang wood. Microemulsions can be used as formulations that can improve the solubility, stability and antioxidant activity of the active compounds (Froelich and Osmałek, 2020). VCO also has several disadvantages, namely the taste of oil and the sticky nature of oil. One way to overcome this is to make VCO into a microemulsion that can reduce the properties of the oil. Therefore, in this study, microemulsion preparations were made from secang wood extract and VCO to be used in various purposes, one of which was cosmetic ingredients.

## RESEARCH METHODS

This study aims to make a stable oil-in-water (o/w) microemulsion of secang wood extract and virgin coconut oil (VCO) with a combination ratio of 3 surfactants with a certain HLB value, as well as analyze the effect of secang wood extract variations on the stability of microemulsions at the same surfactant HLB value. This research will be carried out with 2 main stages. In the first stage, the HLB value of the surfactant mixture is determined which can make the most stable microemulsion. Determination of HLB values was carried out in the range of HLB 12, 13, 14, and 15 with a surfactant:water:VCO:extract ratio of 30:65:2.5:2.5. Stability testing of the resulting microemulsion is carried out through organoleptical observation and turbidity test with storage treatment at room temperature, centrifugation, and heating. The resulting microemulsion was also



tested for the number of free fatty acids as a comparison to pure VCO. Furthermore, the HLB value that produces the most stable microemulsion is used to analyze the effect of secang wood extract variation on the stability of the microemulsion. The analysis was carried out with variations in the water:extract ratio, which is 65.5:2; 65:2,5; 64,5:3; and 64:3,5. The resulting microemulsions were tested for stability through heating and centrifugation through organoleptical observation and turbidity tests, and the most stable microemulsions were tested for antioxidant activity.

## RESULTS AND DISCUSSION

Secang wood is extracted by maceration method to obtain active compounds in the form of flavonoids, especially brazilin. The maceration method was chosen because it is one method that can reduce the damage to active compounds that are not strong heat. In this extraction, 96% ethanol solvent is used because flavonoid compounds in secang wood, namely brazilin have a number of hydroxyl groups that make the compound polar, so it can dissolve in polar solvents such as ethanol.



**Figure 1. Extraction results of secang wood**

Based on the results obtained in Figure 7, the extract obtained at the extraction of 150 g of secang wood in 750 mL of 96% ethanol organoleptic in the form of a reddish-brown, almost odorless viscous liquid. The pH of the extract obtained is 6. The red color of secang wood extract indicates the presence of brazilin compounds in the extract. This result is in accordance with the research of Ngamwonglumlert et al. (2020), brazilin pigments have a red color at neutral pH (pH 6-7), shift towards purplish red with increasing pH, and at low pH (pH 2-5) brazilin has a yellow color.

The presence of brazilin and brazilin in the extract is also strengthened by the results of flavonoid levels from secang wood extract. Quantitative analysis of flavonoid levels from extracts was performed by measuring the absorbance of extracts compared to the standard curve of quercetin solutions. Quercetin is a flavonoid compound with strong antioxidant properties, so it is used as a comparison. The total flavonoid content found in secang wood extract was 23.6% or 230.6 mg QE (quercetin equivalent)/g sample which means in 1 g of extract Secang ethanol contains total flavonoids equivalent to 230.6 mg of quercetin. In the study of Widodo et al. (2022), total flavonoids were obtained in secang ethanol extract of 529.3 mg QE/g samples, while in the study of Purba et al. (2023) total flavonoids were obtained in secang methanol extract of 185.03 mg QE/g samples and total flavonoids in secang water extract were less than methanol extract. This difference in total flavonoids can be caused by different types of solvents used. Based on the results obtained, ethanol solvents are better than methanol and water solvents in extracting secang wood. This can be due to brazilin which is a homoisoflavonoid that is more soluble in ethanol solvents. In the research of Widodo et al. (2022), a higher total flavonoid was obtained from the extraction results even though they used the same solvent. This can be caused by the use of microwave heating-assisted extraction methods in the research of Widodo et al. (2022). Heating

can aid extraction because it facilitates collisions between molecules, so ethanol can more easily extract secondary metabolites from samples.

### Virgin Coconut Oil Fatty Acid Content Analysis

In the analysis of fatty acid content VCO used gas chromatography instruments with mass spectrometry detectors. For volatile substances, it is necessary to derivatize before analysis to increase the volatility of the substance. Fatty acids in VCO have a relatively high boiling point because they have hydrogen bonds in the carboxylate group (-COOH) which causes a high boiling point, so fatty acids need to be derivatized into methyl esters through esterification to lower their boiling point before analysis is performed.

The information obtained from gas chromatography spectra is the retention time of each molecule, while from mass spectrometry spectrometry the relative molecular mass of the sample molecules is obtained. The chromatogram of gas chromatogram analysis on VCO fatty acid derivatives can be seen in Figure IV.2.

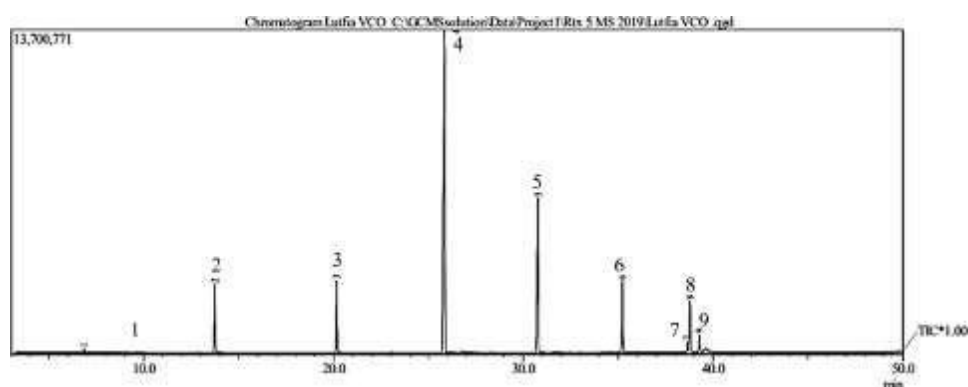
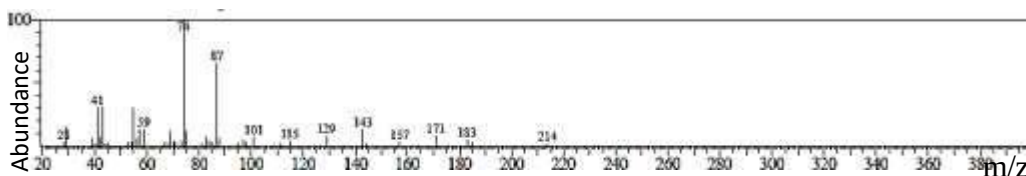


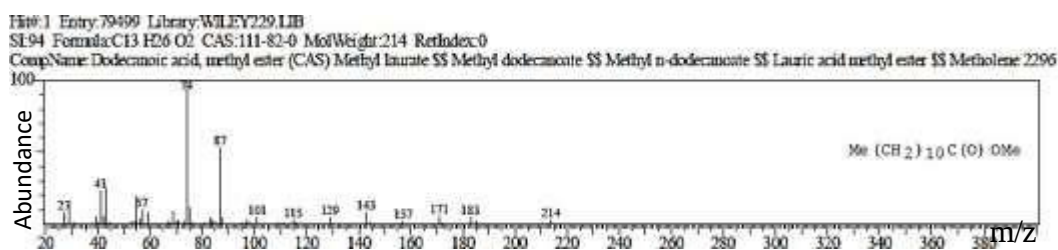
Figure 2. Chromatogram of fatty acid derivatives of VCO

Based on the chromatogram presented in Figure IV.2, 9 peaks were found which indicate that there are 9 compounds contained in VCO. There are 3 peaks with the greatest abundance on the chromatogram, namely peak 4, peak 5, and peak 6. Peak 4 has the greatest abundance of 51.80% with a retention time of 25.849 minutes. Based on mass spectra, peak 4 was identified as fragmentation of dodecanoic acid or methyl lauric ( $\text{Me}(\text{CH}_2)_{10}\text{C}(\text{O})\text{OMe}$ ) with a similarity index of 94% shown in Figure 8.

a)



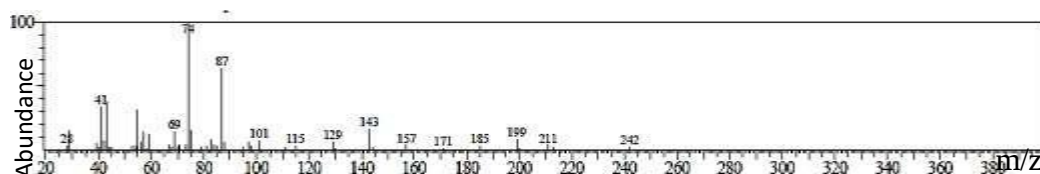
b)



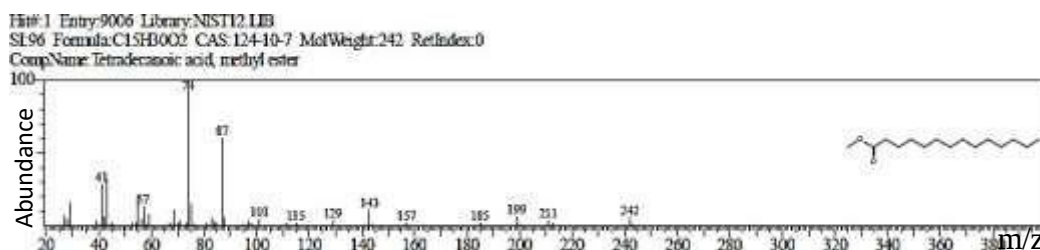
**Figure 3. Mass spectra a) peak 4 fragmentation of VCO derivative results and b) Peak reference spectra 4 (methyl laurate)**

The peak with the second largest abundance is peak 5 which has an abundance of 17.99% with a retention time of 30.765 minutes. Based on mass spectra, peak 5 was identified as fragmentation of tetradecanoic acid or methyl myristate ( $\text{Me}(\text{CH}_2)_{12}\text{C}(\text{O})\text{OMe}$ ) with a similarity index of 96% shown in Figure 9

a)

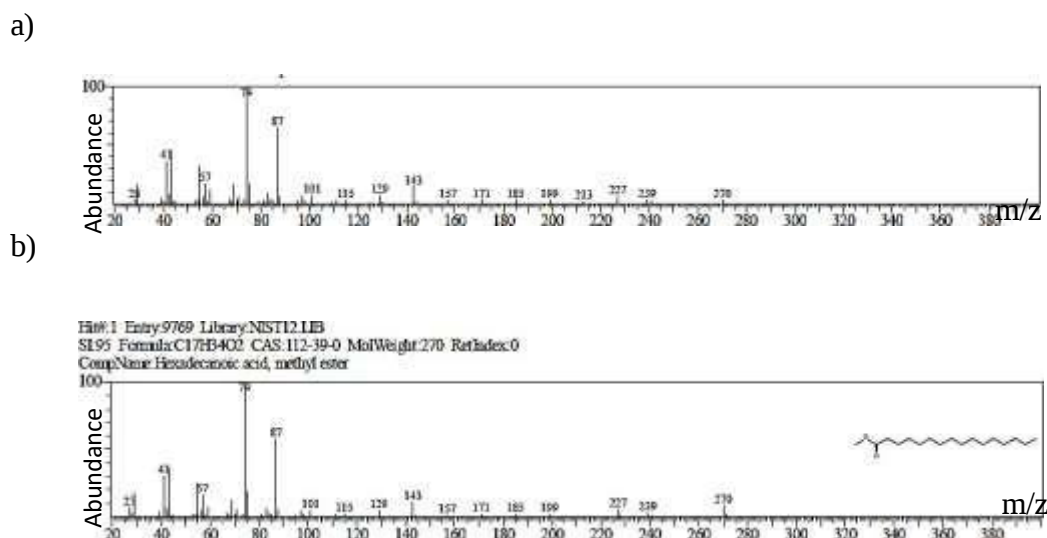


b)



**Figure 4. Mass spectra a) peak 5 fragmentation of VCO derivatives results and b) Peak reference spectra 5 (methyl myristate)**

Peak 6 on the chromatogram is the peak with the third largest abundance at 7.75% with a retention time of 35.217 minutes. Based on mass spectra, peak 6 was identified as fragmentation of hexadecanoic acid or methyl palmitic ( $\text{Me}(\text{CH}_2)_{14}\text{C}(\text{O})\text{OMe}$ ) with a similarity index of 95% as shown by Figure 10.



**Figure 5. Mass spectra a) peak 6 fragmentation of VCO derivatives results and b) Peak reference spectra 6 (methyl palmitate)**

Based on the pattern of mass spectral fragmentation of each peak, it was found that the fatty acid composition contained in VCO can be seen in Table 2

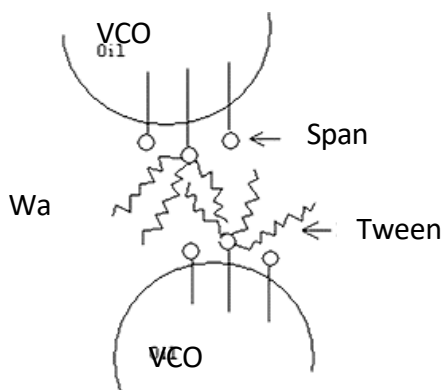
**Table 1.** Chromatogram interpretation of VCO fatty acid derivatives

| Top | Time Retention | % Abundance | Compound Name    | % VCO Composition (APCC, 2009) |
|-----|----------------|-------------|------------------|--------------------------------|
| 1   | 6,847          | 0,34        | Methyl caproate  | 0,10-0,95                      |
| 2   | 13,729         | 6,66        | Methyl caprylate | 4-10                           |
| 3   | 20,158         | 6,96        | Methyl caprate   | 4-8                            |
| 4   | 25,849         | 51,80       | Methyl laurate   | 45-56                          |
| 5   | 30,765         | 17,99       | Methyl myristate | 16-21                          |
| 6   | 35,217         | 7,75        | Methyl palmitate | 7,5-10,2                       |
| 7   | 38,643         | 1,17        | Methyl linoleate | 2-4                            |
| 8   | 38,789         | 5,44        | Methyl oleate    | 4,5-10                         |
| 9   | 39,275         | 1,89        | Methyl stearate  | 0,7-2,5                        |

In gas chromatography, an inert carrier gas in the form of Helium gas is used so that it does not react with the sample and interfere with chromatogram data. The column used in gas chromatography is the DB-5MS column. The DB-5MS column is a phenyl arylene phenyl nonpolar polymer column equivalent to 5% phenyl and 95% dimethylpolysiloxane. The greater the retention time, the more nonpolar compounds contained in VCO. This is in accordance with the chromatogram results that the longer the hydrocarbon chain in the compound, the greater the retention time. Based on data in Table IV.1, there are 2 groups of fatty acids contained in VCO, namely 93.39% saturated fatty acids and 6.61% unsaturated fatty acids. Saturated fatty acids include caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, and stearic acid while unsaturated fatty acids include linoleic acid and oleic acid. Based on the identification of data from the chromatogram, 3 compounds with the greatest abundance were obtained, namely methyl laurate, methyl myristate, and methyl palmitate. The results obtained are in accordance with APCC standards that the 3 compounds with the largest composition in VCO are lauric acid, myristic acid, and palmitic acid. In the interpretation data it can be seen that The main content in VCO is lauric acid with an abundance of 51.80%. Studies prove that the type of fatty acids contained in the oil phase can affect the stability of the microemulsion. According to research by Khor et al. (2014), oils rich in medium-chain triglycerides will produce microemulsions that are more stable and clear than oils with long-chain triglycerides.

#### Microemulsion Formulation with Variation of HLB Value

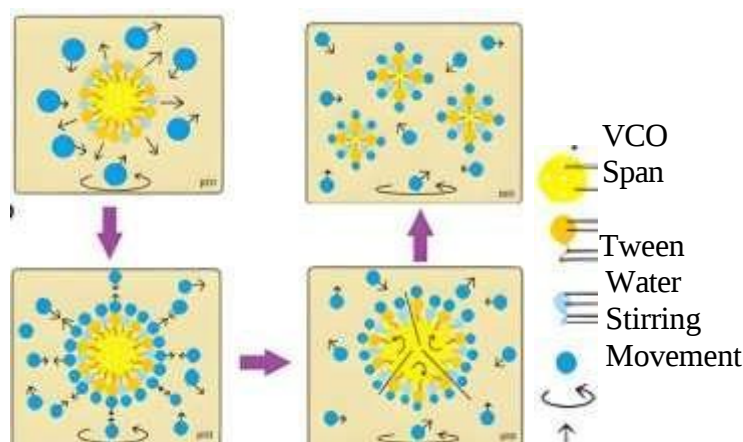
The microemulsion formulation made is an oil-in-water microemulsion using a variety of 3 kinds of surfactants, namely Tween 20, Tween 80, and Span 80 with HLB values of mixtures of 12, 13, 14, and 15. The combination of surfactants with different HLB values can make microemulsions better than single surfactants because the surfactants complement each other's properties. The minimum surface tension that can be achieved is determined by the effectiveness of the surfactant array on the surface and on the interaction between surfactants at the interface. The low molecular weight molecules of Span 80 fill the gaps between the large Tween 80 and Tween 20 surfactants forming a denser arrangement at the oil and water interface that makes the surface tension lower and the interface adsorption higher than that of a single surfactant. In addition, the use of a combination of surfactants is also used to obtain the required HLB because no nonionic surfactant has the appropriate HLB value as required, except for Tween 80 surfactant with an HLB value of 15. Schematically, the interaction between water, surfactant, and VCO can be seen in Figure 11.



**Figure 6. Interaction of surfactants with water interface and VCO**

Chemically, the interaction that occurs in surfactants with water and oil is a non-bonding interaction. The interaction between the hydrocarbon chain of surfactant and oil is the Van Der Waals force, while the interaction at the polar heads of surfactant and water is hydrogen bonding. According to Bayrak and Iscan (2005), the long compatibility of surfactant and oil chains is one of the important factors in the formation of microemulsions. The surfactant in this study has similarities with the oil phase of VCO. Tween 20 surfactants have lipophilic chains in the form of lauric acid which is the largest content in VCO, while Tween 80 and Span 80 surfactants have lipophilic chains in the form of oleic acid which are also contained in VCO. Based on the rule of like dissolve like, a mixture of surfactants with the right ratio will increase the solubility of surfactants, so that the surfactant mixture can penetrate the VCO interface to create clear microemulsions with smaller VCO droplets.

This microemulsion formulation is prepared by mixing the oil, surfactant, and water phases through titration by stirring. Stirring is done with the help of temperature to provide kinetic energy so that collisions between particles occur more and more and form a homogeneous mixture. According to Rao and McClements (2011), there is a kinetic energy barrier in a mixed system when it is first mixed at room temperature that prevents the system from moving to a more kinetically stable state (emulsions and nanoemulsions) or a thermodynamically stable state (microemulsions). As the temperature increases, the kinetic energy barrier in the system decreases which makes the transition possible. This kinetic energy barrier relates to the surface tension between oil and water which is known to decrease considerably as it approaches phase inversion temperature. Heating to a temperature of 55-70 °C can help spontaneous formation of microemulsions. The mechanism of micromulation formation can be seen in Figure 12.



**Figure 7. Mechanism of microemulsion occurrence (Mardiyanto et al., 2018)**

The gradual addition of surfactants, water, and extracts is done to increase the surface area of particles to interact with the mixture, so as to form a stable dispersion system. As stirring progresses, large VCO droplets will split into small droplets characterized by changes in the appearance of the mixture from turbid to clear. After formulation, the mixture is allowed to stand at room temperature to achieve equilibrium. A sign that a microemulsion is successfully formed is that the mixture looks clear or transparent. The small droplet size of the dispersed phase (VCO)

causes the microemulsion not to reflect light and appear transparent. In this study, secang wood extract was added to the mixture, so that the microemulsion formed would look transparent red.

Results obtained from microemulsion formulations with variations in HLB values in the range of 12 – 15 were observed organoleptically including color, odor, clarity, and deposits formed during storage.

#### **HLB 12**

In the manufacture of microemulsions with HLB 12 values, a surfactant mixture consisting of 29.5% Span 80 is used; 5% Tween 20; and 60.5% Tween 80. The high concentration of Span 80 causes the surfactant mixture to become dark brown in color. When adding water with extract to the surfactant mixture with VCO, the mixture forms gel-like lumps. Stirring at 600 rpm for 90 minutes can create lumps. The gel splits and can blend with the water phase to form a light brown viscous mixture. The color of this mixture comes from secang wood extract which is dark red and Span 80 surfactant which is dark brown.

The product obtained is a light brown viscous mixture with an odor like Tween 80. This mixture is an emulsion because it does not look transparent or clear like microemulsions. This can be caused by the high concentration of Span 80 in combination surfactants, thus affecting the increase in viscosity in the emulsion. Span 80 is a nonpolar surfactant that binds oil. The addition of Span 80 as an oil binder which decreases the amount of Tween 80 as a water binder which results in a decrease in surface tension is not optimal which results in large oil droplets. Large oil droplets cause the mixture to look cloudy. The results obtained can be seen in Figure 13.



**Figure 8. Results of microemulsion formulation with HLB 12**

The emulsion looks stable for several days, but on day 3 the emulsion undergoes phase separation. This can be caused by the force of gravity and increased surface tension during storage. VCO droplets fuse with each other (coalescent) due to the breakdown of the protective film layer from surfactants and rise to the surface due to differences in density with water. On the surface of the emulsion there are also surfactant-like spots that do not blend with the mixture.

#### **HLB 13**

In the preparation of microemulsions with HLB 12 values, a surfactant mixture consisting of 21% Span 80 is used; 6.5% Tween 20; and 62.5% Tween 80. The combination of surfactants formed is dark brown because the Span 80 used is still quite high. The addition of extract and water to the mixture of coconut milk with surfactants forms red gel clots. After the addition of the extract

and water is complete, the gel begins to disappear and a brownish-yellow mixture is formed.

The product of making a microemulsion is a non-transparent viscous emulsion with a brownish-yellow color and smelling Tween 80. The high content of Tween 80 causes the smell of secang wood extract to disappear in the mixture. Just like the emulsion on HLB 12, the addition of Span 80 as an oil binder which lowers the amount of Tween 80 as a water binder causes the surface tension cannot be lowered completely. The large concentration of Span 80 in combination with surfactants leads to the formation of a viscous emulsion. The color in HLB 13 emulsion looks browner than HLB 12 emulsion because of the decreased concentration of Span 80 and the color of secang extract dominates. The appearance of the product results can be seen in Figure 14.



**Figure 9. Results of HLB 13 microemulsion formulation**

The resulting emulsion looks stable for several days, but undergoes phase separation after day 4. Oil droplets fuse with each other (coalescent) and separated from the water phase due to the force of gravity and the difference in density between water and oil. The emulsion mixture can become homogeneous again after shaken, but the phases separate again after a while.

#### HLB 14

In the manufacture of microemulsions with HLB 14 values, a surfactant mixture consisting of 13% Span 80 is used; 23% Tween 20; and 64% Tween 80. The mixture of surfactants acquires a light yellow color. The addition of extract and water to the mixture of VCO and surfactant produces a transparent red color without any lumps. After the addition of the extract and water was completed, the mixture looked slightly cloudy, but after constant stirring for 90 minutes a transparent clear mixture was produced which can be seen in Figure IV.10.



**Figure 10. Results of HLB 14 microemulsion formulation**

The resulting product is in the form of a mixture with a transparent red color and a smell like Tween 80. The transparent color of the mixture indicates that the combination of surfactants used can reduce the surface tension between VCO and water to a minimum, so that the resulting VCO droplets become smaller and reflect less light waves. The transparent appearance indicates that the resulting product is a microemulsion. The microemulsion obtained was stable for 3 weeks, but after 4 weeks, a white precipitate was seen indicating phase separation.

#### HLB 15

In making microemulsions with HLB 15 values, a mixture of surfactants consisting of 6% Span 80; 28% Tween 20; and 66% Tween 80 is used. Mixing the extract and water in a mixture of surfactant and VCO produces a mixture that is lighter red than the mixture in HLB 14. This can be caused by a decrease in the concentration of Span 80 which gives the mixture a brown color. At first the mixture looks cloudy, but after constant stirring, it becomes clear and transparent as presented in Figure IV.11.



**Figure 11. Results of HLB 15 microemulsion formulation**

The result obtained is a microemulsion with a transparent dark red color and smells like Tween 80. The color of HLB 15 microemulsions looks older than HLB 14 microemulsions due to

the addition of Tween 80 and the reduction of Span 80 in their formulation. The microemulsions formed showed that the combination of HLB 15 surfactants can lower the surface tension to a minimum. Microemulsions appear stable for up to 4 weeks without any phase separation and deposits in the system.

## CONCLUSION

Based on the results and discussion of the research that has been done, it was concluded that: Extraction of 150 g of secang sawdust in 750 mL of 96% ethanol produced secang extract which has a reddish-brown color. The total flavonoids in secang extract were 23.6% or equivalent to 230.6 mg QE/g sample. The most stable oil-in-water microemulsions are microemulsions with HLB Microemulsions formulated with 2.5 mL VCO; 2.5 mL secang extract; 65 mL of water; and 30 mL of surfactant mixture with a composition of 6 mL Span 80, 66 mL Tween 80; and 28 mL Tween 20. Microemulsions appear transparent red, 1 phase, and smell like Tween 80 with turbidity values below 1% during storage, heating, and centrifugation. The higher the concentration of secang extract, the stability of the microemulsion is reduced. The most optimal concentration of secang extract that produces stable microemulsions is microemulsions with 3% secang extract. The microemulsion has antioxidant activity with an IC<sub>50</sub> value of 1.4573±0.2694 mg/mL.

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