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Synthesis of Red Fruit Oil (Pandanus Conoideus) Emulsion with Tween 80 Surfactant and Alginate Co-Surfactant

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ABSTRACT

Red fruit (pandanus conoideus) is one of the natural biological resources that contains lipid compounds that are beneficial and important for health. However, the content of red fruit oil components is sensitive to oxygen, light, and heat, so it impacts damage to the content and a relatively short shelf life. The emulsification method can increase the benefits and shelf life of red fruit oil. Emulsions can be stored by preserving active substances in their core and protecting them with a shell layer. Adding alginate to the water phase can increase the stability of the emulsion against aggregation because these molecules can cause steric and electrostatic repulsion between droplet interfaces. This study aims to synthesize red fruit oil emulsions with alginate as a co-surfactant. The variables observed were the ratio of ingredients, the effect of speed, and the time of emulsification stirring. The study's results, namely alginate, can be used as a co-surfactant in the synthesis of red fruit oil emulsions. The ratio of red fruit oil emulsion ingredients is 1% weight/volume, tween 80 1% weight/volume, and alginate 2% weight/volume of a total volume of 40 ml. The emulsification process conditions were carried out at room temperature with a stirring speed of 25,000 rpm and a stirring time of 10 minutes. The resulting emulsion is an oil-in-water emulsion (m/a). The emulsion is dominated by hydrophilic or polar components caused by tween 80 surfactants and alginate co-surfactants. In addition, the creaming formation time occurred after 216 hours, and the separation time occurred after storage for 552 hours.

Keywords: alginate, biolipid, emulsion, red fruit oil, tween 80.

1. INTRODUCTION

Biological natural resources are sources of chemical compounds that are unlimited in type and quantity, so biodiversity can be interpreted as chemical diversity that can produce chemicals for the needs of humans and other organisms, such as medicines, insecticides, and cosmetics[1-3]. Red fruit (pandanus conoideus) is a biological natural resource that contains bioactive lipid compounds (biolipids), which are beneficial and essential for health. The red fruit plant (Pandanus conoideus) belongs to the Pandanaceae family and is endemic to Papua Island. Research shows that red fruit extract oil is safe for human consumption, inhibits tumour growth, kills cancer cells, and cures malaria [4-7]. In addition, red fruit provides anti-inflammatory activity and improves the immune system [8]. The chemical compounds contained in red fruit include oleic, linoleic, linolenic, and palmitoleic acids, as well as various active minor components, including α-carotene, β-carotene, β-cryptoxanthin, α-tocopherol and phenol components [9-12].

The result of extracting red fruit is crude oil, which contains non-triglyceride components

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(phospholipids or sticky protein-fat and carbohydrate complexes such as sap and mucus (gum), pigments, heavy metals, and free fatty acids) and has hydrophobic properties so that its use in food is still limited [13,14]. Apart from that, the carotene content of red fruit is sensitive to oxygen, light, and heat [15], resulting in damage to the content and a relatively short shelf life. Emulsification is a viable method to enhance the stability and extend the shelf life of red fruit oil by protecting its bioactive components from environmental stressors.

Emulsion is a colloidal dispersion produced from water, oil, and surfactants. Emulsions have been widely used in biological, food, and pharmaceutical applications because they can function as carriers or delivery systems for bioactive ingredients and lipophilic compounds in functional foods, cosmetics, and pharmaceutical products. To obtain an emulsion, a surfactant is needed to absorb at the droplets' oil-water interface, provide a flexible film layer around the droplets and reduce surface tension [16]. Surfactants can reduce interfacial tension, which will help the dispersion process in the emulsion system and form a flexible film layer to break up the dispersed phase droplets [17]. Tween 80 is a nonionic surfactant type emulsifier with the advantages of being non-toxic and non-irritating [18] and is most widely used for emulsification [19]. In addition, tween 80 is suitable for forming oil/water emulsions because it can produce steric stability and keep the droplets separated through steric hindrance [20,21].

Polysaccharides, such as alginate, can be added in the aqueous phase of the emulsion [16,19,22]. The addition of alginate to the aqueous phase can improve the stability of nanoemulsions against aggregation because these molecules can cause steric and electrostatic repulsion between the droplet interfaces [16], preventing destabilization caused by droplet coalescence or gravitational separation. Functional groups in alginate, such as carboxylates, easily dissociate in the aqueous phase and impart a negative charge to the emulsion [16,19,22]. In addition, the interaction of tween 80 and alginate affects the surface charge of lipid droplets. However, adding alginate to the aqueous phase must be carefully controlled because it can have either a positive or negative effect on the physical stability of the emulsion [19,23].

Based on the description above, this study aims to synthesize red fruit oil emulsion with alginate as a co-surfactant and tween 80 surfactant. The parameters used are the ratio of alginate, red fruit oil, and tween 80. In addition, stirring speed and time affect red fruit oil emulsification.

2. RESEARCH METHODS

2.1. Materials and Tools

The materials used in this study were red fruit oil (pandanus conoideus) produced by Sinar Baliem Wamena, alginate and tween 80 with food-grade specifications purchased from PT. Multi Jaya Kimia, and distilled water. The tools used were a Sonifer SF-8044 stirrer (220-240V, 50/60Hz, 500W), beaker glass, analytical balance, and thermo scientific genesys 50 UV-Vis Spectrophotometer. Fourier Transform Infra-Red (FTIR): Perkin-Elmer UATR Spectrum Two.

2.2. Functional Group Test

The functional group test is a qualitative analysis that functions to determine functional groups using the Fourier Transform Infrared Spectroscopy (FTIR) instrument.

2.3. Emulsification of Red Fruit Oil

Red fruit oil emulsion samples were prepared by separating the oil and water phases. The oil phase consisted of 0.4 grams of red fruit oil, 0.4 grams of tween 80, and 10 ml of distilled water from 40 ml, then stirred for 1-2 minutes. The ratio of red fruit oil and tween 80 remained (unchanged). The water phase was made by dissolving alginate (0.4, 0.8, 1.2) grams in 30 ml of distilled water and stirring for 2 minutes. In the next stage, the oil phase was added to the water phase and stirred at speed (1,500 rpm and 25,000 rpm) and stirring time (1; 5; 10) minutes. After emulsification, the sample analysis was carried out using the emulsion type, stability, and transmittance tests.

2.4. Emulsion type testing

Emulsion-type testing with a colour method using methyl blue-soluble dye. Testing can be diluted based on the outer phase of oil-in-water (o/w) emulsion. Drip the emulsion into a watched cup, add 1-2 drops of methyl blue to the cup sample and see the colour change. The addition of methyl blue will give a blue colour evenly to the o/w type emulsion [24].

2.5. Emulsion Stability Test

The cycling test method functions to observe the stability of the emulsion, namely by storing each sample at a temperature of 4 ± 2°C for 24 hours and then moving it to room temperature for 24 hours (1 cycle). Observations are made visually by observing colour, clarity and phase separation using the five senses at each cycle. After the sample has gone through the cycling test, it is shaken lightly for 1 minute to observe whether the emulsion is reversible or irreversible [25].

2.6. Transmittance Test

The transmittance test functions to determine the clarity of the emulsion. Emulsion clarity is an indicator of complete dispersion. The tool used was UV-Vis spectrophotometry with a wavelength of 650 nm [4].

3. RESULTS AND DISCUSSION

3.1. FTIR (Fourier Transform Infra Red) Analysis of Red Fruit Oil

FTIR analysis aims to identify chemical bonds or functional groups in red fruit oil. The results of FTIR analysis of red fruit oil show that there are functional groups consisting of organic compounds such as esters, carboxylic acids, and unsaturated hydrocarbons. The FTIR absorbance spectrum measurement results of red fruit oil are as follows: at a wave number of 3000 cm^{-1}, a =C-H stretch bond with an alkene functional group was detected. Alkenes are groups commonly found in vegetable oils with many unsaturated fatty acids [3]. Similar wave numbers were also detected in sunflower and corn kernel oils, namely at 3006-3011 cm^{-1}, due to the content of unsaturated fatty acids, especially linoleic acid [26]. The absorption of wave numbers 2922 cm^{-1} and 2854 cm^{-1} shows symmetric and asymmetric stretching vibrations ν(C-H) of the aliphatic group CH_2. The same absorption frequency occurs in olive and palm oils, 2924 cm^{-1} and 2852 cm^{-1} [9]. The C=O stretch bond with the ester functional group is shown at a wave number of 1744 cm^{-1}. The ester functional group with the C-O stretch bond is also seen at a wave number 1166 cm^{-1}. The ester bond indicates the presence of glycerol, which is still bound to fatty acids. Similar wave numbers are also detected in olive oil (1746 cm^{-1}), palm oil (1741 cm^{-1}), and coconut oil (1743 cm^{-1}) [3,9,26]. Bending vibrations of the CH_2 and CH_3 aliphatic groups are seen at wavenumber 1465 cm^{-1}. The C-H bond is seen at a wave number of 725 cm^{-1}. At the same time, in palm oil and olive oil, the absorption of 722 cm^{-1} shows the overlapping of the methylene ($-CH_2$) rocking vibration and the out-of-plane vibration of cis-disubstituted olefins [9]. The FTIR Wave Number and Absorbance of Red Fruit Oil are presented in Table 1 and Figure 1.

3.2. Emulsion Type Test

The emulsion type test is carried out by mixing the resulting emulsion with methylene blue on a watch glass, then stirring and observing visually. Methylene blue is a dye because it is soluble in water and produces a homogeneous colour. These characteristics are suitable for testing the oil-in-water (o/w) emulsion type [27]. One of the signs that the red fruit oil emulsion is stable and well homogenized is when the colour changes from initially dark red to orange-red (Figure 2).

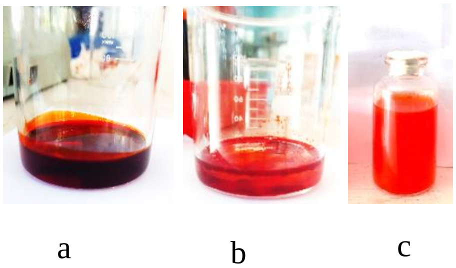

Figure 2. a) Red fruit oil; b) red fruit oil with water; c) Emulsification of red fruit oil.

Based on the test results, this research sample is an o/w emulsion type. It can be seen in Figure 3 that methylene blue dissolves completely, produces a homogeneous blue colour, and does not clump. This is because hydrophilic or polar components dominate the emulsion sample due to the use of tween 80 surfactant and alginate co-surfactant. Therefore, polar methylene blue can dissolve and spread evenly in the sample. However, methylene blue will clump on the surface in the water-in-oil (w/o) emulsion type because the emulsion is dominated by hydrophobic or non-polar components [27,28]. Hydrophilic-lipophilic balance (HLB) is the balance of size and strength of the hydrophilic and lipophilic groups of a surfactant molecule. The HLB scale ranges from 0 to 20. In the range of 3.5 to 6.0, surfactants are more suitable for use in o/w emulsions. Surfactants with HLB values in the range of 8 to 18 are most commonly used in o/w emulsions [29,30]

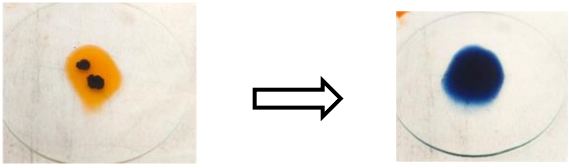

Figure 3. Sample with methylene blue.

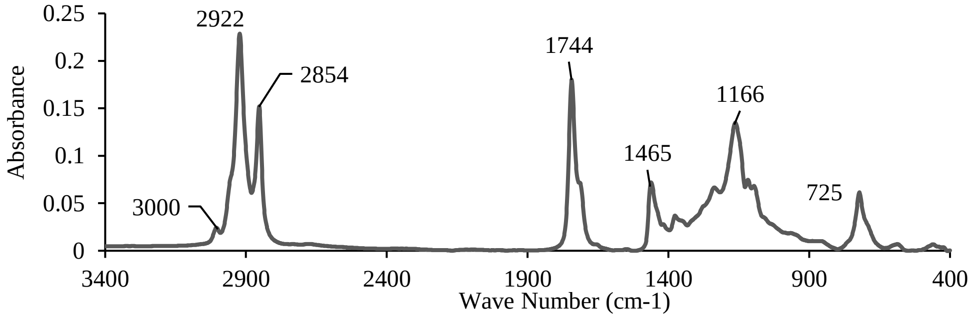

Figure 1. FTIR Analysis of Red Fruit Oil.

Table 1. Wave Number and FTIR Absorbance of Red Fruit Oil.

Functional Groups	Red Fruit Oil	
	Wave Number (cm^{-1})	Absorbance
• =C-H (alkene)	3000	0.023
• Asymmetrical and symmetrical stretching vibration of methylene ($-CH_2$) group	2922 and 2854	0.229 and 0.147
• C=O stretch	1744	0.175
• Bending vibrations of the CH_2 and CH_3 aliphatic groups	1465	0.068
• Stretching vibration of the C-O ester group	1166	0.133
• C-H (long chain methyl)m	725	0.059

3.3. Effect of Stirring Speed

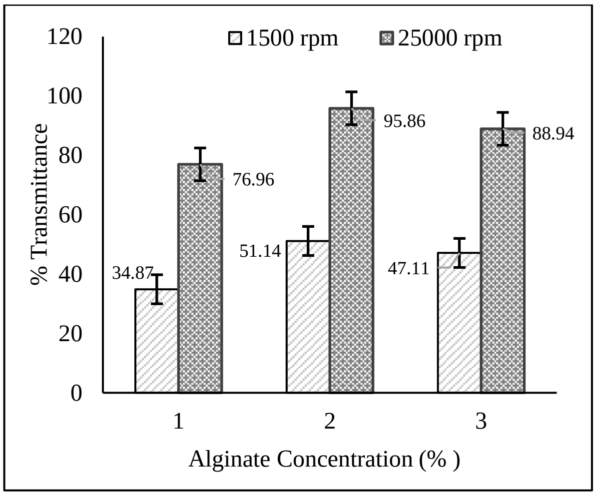

Figure 4. Graph of the Effect of Stirring Speed.

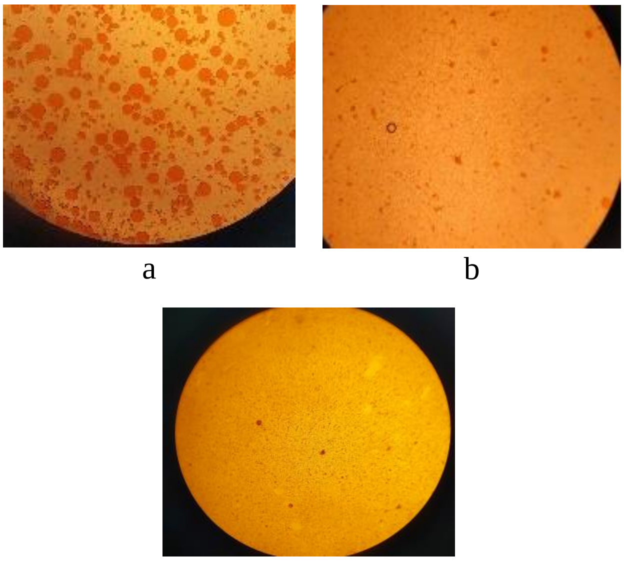

Figure 5. Red fruit oil droplet molecules: a) before stirring the oil phase; b) emulsion stirring 1500 rpm; c) emulsion stirring 25000 rpm.

Figure 4 shows a graph of the effect of stirring speed and alginate concentration on the transmittance value. The highest transmittance value was obtained at an alginate concentration of 2% with a stirring speed of 25000 rpm, which was 95.86% for 10 minutes. The percentage of transmittance value> 90% and approaching 100% indicates that the emulsion particle size is tiny so that it can pass through the light beam, and the dispersion is visually apparent [31]. This shows the formation of an emulsion capable of producing small particle sizes, as seen in Figure 5. Figure 5 shows the red fruit oil droplet molecules before stirring and the emulsion droplets at different speeds. Before mixing the red fruit oil droplet molecules, they were homogeneous and not the same size (Figure 5a). At 1500 rpm stirring, a smaller particle size was obtained than before, but it was not homogeneous because some large droplets were still estimated to be red fruit oil (Figure 5b). Figure 5c shows a smaller, more homogeneous particle size obtained at 25000 rpm stirring.

Stirring speed affects emulsions' formation and the emulsion stability level [32]. Increasing the stirring speed causes particles to collide with each other. It can expand the contact area, thereby increasing the homogeneity of an emulsion, while stirring that is too slow produces an inhomogeneous emulsion [33,34]. Wiyani et al.'s research [35] synthesized virgin coconut oil-orange juice emulsions with gum arabic emulsifier at various stirring speeds of 5000, 10000, and 15000 rpm. Stable emulsion products at room temperature were obtained at a speed of 15000 rpm. Similar results were obtained in the study of citronella oil nanoemulsions, which were stirred at 12000 rpm. This resulted in stable nanoemulsions for ten days of storage, namely no phase separation in the nanoemulsion system [36]. Stirring or agitation is a process that shows induced movement in a material or mixture that will form a circulation pattern to affect the homogenization process [35,36]. The stirring speed will reduce the viscosity of the emulsion formed. In addition, stirring can reduce interfacial tension and expand the surface of the globule [37].

In addition to the effect of speed, alginate concentration variation is also shown in Figure 4. At 1% alginate concentration, it gave a smaller transmittance value (34.87% and 79.96%) compared to 2% alginate concentration (51.14% and 95.86%) and 3% (47.11% and 88.94%) with the same stirring speed. Similar results in the study of Hosseini et al. [38] verified higher droplets with increasing polysaccharide concentration. At the same time, Salvia-Trujillo et al. [39] observed that increasing SA concentration from 0.5 to 1.0% resulted in a reduction in droplet size. These effects were associated with thinning, flocculation, and coalescence of droplets at lower concentrations, while polysaccharides protected the droplets from coalescence at higher alginate concentrations. In addition, tween 80 and alginate interaction affected the surface charge of lipid droplets [16].

3.4. Effect of Mixing

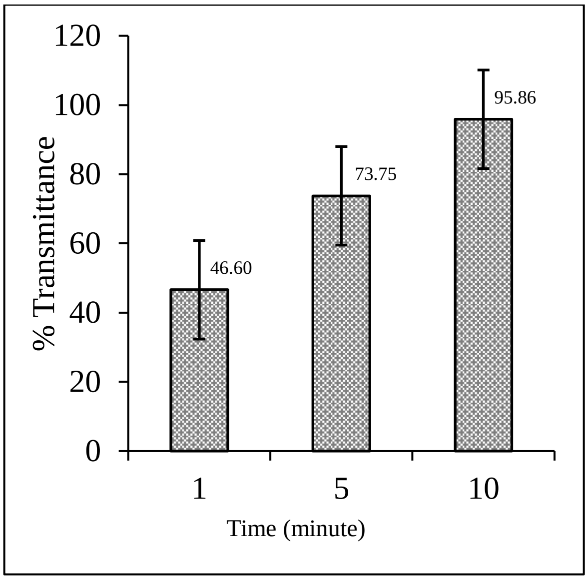

Figure 6. Graph of the Effect of Stirring Time.

The stirring time affects the emulsion results presented in Figure 6, showing that the stirring time for 10 minutes produces a transmittance value of 95.86% at an alginate concentration of 2%. The standard deviation for the three data in Figure 6 is 18.01, 1.19, and 16.82, with an overall data average of 72.07. The standard deviation of each data has a value smaller than the average value, which means that the data obtained has a slight variation. Meanwhile, the effect of stirring time, namely, the longer the stirring time, the longer the oil-in-water emulsion's separation time and the emulsion's stability increases [36,40]. In this study, the stability of the emulsion can be seen from the transmittance value because the higher the transmittance value, the smaller the particle size, with a visually apparent dispersion.

Table 2. Creaming Formation Time.

Sample	Mixing Time (Minutes)	Creaming formation time (Hours)	Appearance
1	1	48	Murky orange and thick creaming layer
2	5	168	Orange and thin creaming layer
3	10	216	Clear orange and thin creaming layer

Table 3. Storage Time.

Sample	Mixing Time (Minutes)	Storage Time (Hours)	Appearance
1	1	360	The murky orange begins to fade, thick creaming layer, and separate phases
2	5	480	The murky orange begins to fade, thin creaming layer and phase-separated
3	10	552	Clear yet murky orange, the creaming layer is thin, and the phases are not perfectly separated

Stirring time also visually affects the creaming formation and storage time, as seen in Table 2 and 3. It can be seen in Table 2 that the emulsion with a stirring time of 1-minute experiences creaming faster, namely at 48 hours, while the stirring time of 5 minutes and 10 minutes, the emulsion tends to be stable or a thin creaming layer is formed at 168 hours and 216 hours. However, when lightly shaken, the sample will mix again (reversible). Table 3 presents the effect of stirring time on storage time. The faster the stirring time, the longer the storage time of the emulsion product cannot last. This can be seen at a stirring time of 1 minute; the emulsion product experiences separation with the orange emulsion colour fading, and the phase is separated during a storage time of 360 hours. However, on the contrary, a stirring time of 10 minutes shows the emulsion product is still clearly orange but cloudy, and the creaming layer and the phase have yet to separate, with a storage time of 552 hours completely.

Emulsions that have a homogeneous appearance will be more stable because there are no lumps caused by the flocculation structure of small emulsion globules that trap a large number of dispersing phases or water phases that will accelerate the occurrence of sedimentation [25,41]. Sedimentation in emulsions is caused by the difference in density between the two emulsion phases; if the dispersing phase is denser than the dispersed phase, it will form creaming upwards, while if the dispersed phase is denser than the dispersing phase, it will form creaming downwards [42]. Several factors cause instability in an emulsion, such as emulsifier concentration, oil and water concentration ratio, temperature, and stirring time [43].

4. CONCLUSION

Based on the study's results, it can be concluded that alginate can be used as a co-surfactant in synthesising red fruit oil emulsions. In this study, the stability of the emulsion was seen from the transmittance value. The higher the transmittance value, the smaller the particle size because the emulsion product produces a visually apparent dispersion. The highest transmittance value was 95.86% with a formulation of 1% red fruit oil weight/volume, surfactant (tween 80) 1% weight/volume, and alginate 2% weight/volume from a total volume of 40 ml. The emulsification process conditions were carried out at room temperature with a stirring speed of 25,000 rpm and a stirring time of 10 minutes. The resulting emulsion is an oil-in-water (o/a) emulsion. This is because the emulsion is dominated by hydrophilic or polar components caused by tween 80 surfactant and alginate co-surfactant. In addition, the creaming formation time occurred after 216 hours, and the separation time occurred after storage for 552 hours.

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