Food Research 6 (4) : 120 - 127 (August 2022)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Formulation and stability of *Ulva lactuca* fatty acid oil in water (O/W) microemulsion

Ningrum, Y.P. and *Budhiyanti, S.A.

Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia

Article history:
Received: 2 July 2021
Received in revised form: 3 August 2021
Accepted: 15 November 2021
Available Online: 14 July 2022

Keywords:
Ulva lactuca,
Fatty acids,
Oil in water,
Microemulsion formulation,
Stability

DOI:
https://doi.org/10.26656/fr.2017.6(4).575

Abstract

The research was conducted to produce stable oil in water microemulsion (O/W) of *Ulva lactuca* fatty acid. *Ulva lactuca* green algae samples were taken from Ngandong Beach, Gunungkidul. This research consisted of several stages, including the extraction of *U. lactuca* fatty acids, and microemulsion formulations made of *U. lactuca* fatty acid microemulsions. The tests carried out include turbidity index, heating, centrifugation, electrical conductivity, and peroxide value. *Ulva lactuca* fatty acid extraction was carried out using ethanol-hexane at 70°C for 3 hrs, and then the extract was stored in the freezer until used. Microemulsion formulation was made of variations of Tween 80, Span 80, and Tween 20 surfactants with 0 to 90% water content at 70°C. All formulas were then tested for heating, centrifugation, turbidity index, and electrical conductivity. The results showed that stable oil in water microemulsions was the sample with a water content of 70, 80, and 90% of each formula. The stabilized microemulsions were added with *U. lactuca* extract with 100, 200, and 300 ppm concentrations, then tested for centrifugation, heating, and turbidity index. The most stable formula was tested for peroxide value. The two microemulsions with the best results were the formula of A2-200 (Tween 80: Span 80: Tween 20 = 92: 5.5: 2.5, v/v) and C2-200 (Tween 80: Span 80: Tween 20 = 87: 5.5: 7.5, v/v) with 80% water content and 200 ppm of *U. lactuca* fatty acid extract.

1. Introduction

Macroalgae are eukaryotic, multicellular organisms found in intertidal areas with sufficient sunlight and attach to substrates. Macroalgae can grow in all waters in Indonesia. According to Safia (2013), a coastal area in Gunungkidul Regency, Yogyakarta, Indonesia has 39 species of macroalgae, consisting of 7 members of Chlorophyceae, 4 members of Phaeophyceae, and 28 members of Rhodophyceae. Macroalgae have a low-calorie content but are high in minerals, vitamins, protein, and carbohydrates. Although the lipid content in macroalgae is low, polyunsaturated fatty acids (PUFA) in macroalgae are high, mainly C18 and C20 (Kumari *et al*., 2011). PUFA plays an essential role in fat transport and metabolism, immune function, and cell membrane function (Ginsberg and Karmally, 2000; Schmid *et al.,* 2014).

Ulva lactuca is a green macroalga or Chlorophyta that has a high density on the coast of the Gunungkidul region, Yogyakarta, Indonesia, which is a good source of PUFA (Yaich *et al.,* 2011; Handayani, 2016). Saraswati (2020) revealed that *U. lactuca* taken from Ngandong Beach, Gunungkidul, contained palmitic acid, stearic acid, linolenic acid, and oleic acid. Chlorophyta fatty acids, especially *U. lactuca* have not been widely used in the food industry because they are hydrophobic, and thus, cannot be dissolved in water properly. According to Flanagan and Singh (2006), one way to increase a hydrophobic substance's solubility is to make it an emulsion preparation. The emulsion system can increase the solubility of hydrophobic substances. One form of development of the emulsion preparation system is microemulsion. The microemulsion is a stable, homogeneous, and transparent thermodynamic dispersion system with particle sizes ranging from 5 nm to 100 nm. This system consists of water, oil, and surfactant phases with different ratios, and cosurfactants are usually added (Cho *et al.*, 2008; Abbasi and Radi, 2016; Xu *et al.*, 2016). Lawrence and Rees (2012) explained that microemulsion is a colloidal dispersion system consisting of oil or water on a micro-scale stabilized by surfactants and has stable, transparent, isotropic, and low viscosity thermodynamic characters. The character is also an excess of microemulsions than ordinary emulsion preparations.

*Corresponding author.
Email: *sitabudhiyanti@ugm.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

Microemulsion has high stability with suitable composition or ratio of surfactant, water, and oil, the formulation process is needed to get the best composition (Burguera and Burguera, 2012; Xu *et al*., 2016). Microemulsion with the best stability is expected to facilitate *U. lactuca* fatty acid extract into a food product. Research conducted by Sary (2019) and Safitri (2019) examined the microemulsion of *U. lactuca* fatty acid extracts with varying concentrations of fatty acid extracts and their stability in application to beverage products. The study only used one surfactant formula from the best formulation from Suhendra's research (2012) using VCO and fucoxanthin compounds, that is why research is needed related to variations in the type and concentration of surfactant, water, and *U. lactuca* fatty acid extracts. This research was conducted to determine the variation and concentration of surfactants in the production of *U. lactuca* fatty acid microemulsion to be applied to a broader range of products.

2. Materials and methods

2.1 Materials

Ulva lactuca were harvested and collected from Ngandong Beach Gunungkidul in March 2019. The sample transport was carried out in cold temperatures using insulated boxes. After arriving in the laboratory, the samples were cleaned using running water and stored in the freezer at a temperature of -10^{o}C until used.

The chemicals used for fatty acid extraction and analysis were sodium sulfate (Na_2SO_4), sodium hydroxide (NaOH), chloroform, HCl, methanol, Tween 20, Tween 80, acetic acid, Iron (II)chloride from Merck, Span 80, acetyl chloride, nonadecanoic acid (C19:0), ammonium thiocyanate from Sigma-Aldrich, phosphate buffer pH 7, hexane, ethanol, nitrogen (N_2), distilled water.

2.2 Fatty acid extraction

The fatty acid extraction was done according to Kumari *et al.* (2011) and Saraswati (2020). Fresh *U. lactuca* (1 g) was added with 10 mL of hexane: ethanol (1: 19, v/v). The samples were vortexed for 1 min and then heated at 70^{o}C with a water bath shaker for 3 hrs, at a speed of 100 rpm. The sample was then cooled and added 2 mL of water and 4 mL of hexane, then centrifuged at 3000 rpm for 7 mins. The organic phase was collected, and the water was removed with Na_2SO_4. The extract was then evaporated with nitrogen, and the fatty acid extract was obtained. The extract was then stored at -20^{o}C.

2.3 Determination of surfactants and water ratio

Microemulsion formulation referred to the technique of making microemulsion without co-surfactant by Suhendra *et al.* (2012). Microemulsion composition consisted of oil, surfactants, and water. The surfactants consisted of two types, namely hydrophilic surfactants (Tween 20 and Tween 80) and lipophilic surfactants (Span 80), with Hydrophilic Lipophilic Balance (HLB) (v/v/v) 14.5. The HLB 14.5 was based on Suhendra *et al.* (2012), which showed the HLB value had the best stability among the others. The HLB was made with three different formulas. The surfactant formulation was mixed with water dropwise to have a 0-90% water concentration, as presented in Table 1.

The surfactant-water mixtures were made by adding water dropwise to 10-90% water content at a temperature of 70±5°C with a magnetic stirrer (100 rpm). The microemulsions formed were then tested for stability after 24 hrs of incubation. The stability tests were carried out by turbidity test after centrifugation at 3000 rpm for 20 mins and heating at 105°C (5 hrs). The turbidity was observed with a UV-Vis wavelength of 502 nm (Cho *et al.,* 2008). Measurement of microemulsion conductivity was carried out 24 hrs after incubation and used the distilled water to compare (Suhendra *et al.*, 2012).

2.4 Production of Ulva lactuca fatty acid microemulsions

Preparation of *U. lactuca* fatty acid microemulsion

Table 1. Formulation of Microemulsion with Tween 80 (T80), Span 80 (S80), and Tween 20 (T20) as surfactants

Formula A T80:S80:T20 = 92:5.5:2.5			Formula B T80:S80:T20 = 89.5:5.5:5			Formula CC (T80:S80:T20 = 87: 5.5: 7.5		
Code	Surfactant (%)	Water (%)	Code	Surfactant (%)	Water (%)	Code	Surfactant (%)	Water (%)
A1	10	90	B1	10	90	C1	10	90
A2	20	80	B2	20	80	C2	20	80
A3	30	70	B3	30	70	C3	30	70
A4	40	60	B4	40	60	C4	40	60
A5	50	50	B5	50	50	C5	50	50
A6	60	40	B6	60	40	C6	60	40
A7	70	30	B7	70	30	C7	70	30
A8	80	20	B8	80	20	C8	80	20
A9	90	10	B9	90	10	C9	90	10

Source: Suhendra *et al.* (2012)

was carried out by adding 100, 200, and 300 ppm *U. lactuca* fatty acids to a stable surfactant-water mixture. The mixture was then mixed with a magnetic stirrer at 70±5°C and 100 rpm. Microemulsion stability testing was carried out after 24 hrs of storage, including the visual appearance and turbidity test after centrifugation at 3000 rpm for 20 mins and after heating at 105°C (5 hrs). According to Cho *et al.* (2008), oil in water (O/W) microemulsion is stable if there were a transparent appearance, no gel formed, and a turbidity index of less than 1%.

Measurement of the microemulsion turbidity index was performed with a UV/VIS spectrophotometer at a wavelength of λ 502 nm with the formula :

Turbidity (%) = absorbance × 2.303/cuvette width (cm)

2.5 Peroxide value (PV) analysis

The determination of peroxide value was carried out by the Hills and Tiel Ferric Thiocyanate method, which was approved by Chapman and Mackay in Adnan (1980) using methanol as a solvent. A sample of 50 µL was put into a screw cap test tube, and then 10 mL of methanol, 50 µL ammonium thiocyanate, and 50 µL ferrous chloride or Fe(II) chloride was added. The test tube was tightly closed, shaken in a water bath shaker at 50°C for 2 mins, and then cooled at 25°C. The formation of red colour was analysed with a spectrophotometer at a wavelength of 510 nm. The blank was done as in the sample. The peroxide number showed in mill equivalent per kg of oil (mEq/kg) was formulated as follows:

$$\text{Peroxide value (meq/kg)} = \frac{A \times B \times DF}{C \times 55.84}$$

Where A is the amount of µg Fe in 10 mL, B is solution volume (10 mL), C is samples weight (g), 55.84 is correction factor and DF is the dilution factor

2.6 Experimental design

The experimental design used was a completely randomized design (CRD) with three replications. The data obtained were analyzed using analysis of variance (ANOVA). If there was a significant difference, Tukey's test was performed to determine the difference between each treatment at a significance level of 95%. Statistical analysis of the data was processed using SPSS 20 software.

3. Results and discussion

3.1 Preparation of stable microemulsions

Microemulsions were stable, homogeneous, and transparent thermodynamic dispersion systems with particle sizes ranging from 5 nm to 100 nm. This system consisted of water, oil, and surfactant phases with different ratios and usually added cosurfactants (Cho *et al*., 2008; Abbasi and Radi, 2016; Xu *et al.*, 2016). Microemulsions had high stability and solubility when the correct surfactant type and ratio are obtained. The appropriate ratio or formulation for making stable microemulsions could be achieved by mixing surfactants with various hydrophilic-lipophilic balance (HLB). Microemulsions consisting of surfactants with different HLB numbers (high/medium/low) would form tiny particles that can increase solubility (Suhendra *et al.,* 2014). This study used surfactants, which each had different HLB values, namely Tween 20 with HLB 16.7, Tween 80 with HLB 15, and Span 80 with HLB 4.3. Microemulsion stability could be measured by conducting several tests, including turbidity, conductivity, heating, centrifugation, and peroxide values. In general, microemulsions were called stable if they appear transparent, do not form a gel, and have a turbidity index of less than 1% (Cho *et al*., 2008; Xu *et al.,* 2016).

3.1.1 Turbidity and appearance test

Turbidity and visibility tests were performed on all samples, with various compositions of 3 types of surfactants, namely Tween 20, Tween 80 Span 80. Each surfactant formula was varied again with the percentage of water. The microemulsion was stored for 24 hrs then turbidity and appearance were observed. Observations of the sample's physical appearance showed that all samples had a clear appearance and were without sediment. The sample's turbidity level increased from sample A1 to sample A4, the gel formed at A5, and turbidity increased from A6 to sample A10. The same thing happened to the samples formulas B and C. Formulas A5, B5 and C5 had the highest viscosity, which caused the samples to form a very consistent gel so that after the centrifugation test, the samples did not allow for the turbidity test.

The thickening phenomenon was explained by Luisi *et al.* (1990) that under certain conditions, the microemulsion could turn into a very thick gel known as an organogel. Sagiri *et al.* (2012) explained that Span and Tween could form organogel or structures like fibres, which physically interact with each other and form the organogel's network structure. In Table 2, the gel phase in samples A5, B5, and C5 slightly melted after the heating test but formed a gel again after the temperature cooled. This condition can be explained by Haering and Luisi (1986), the formation of gel in microemulsions was reversible, heating above 40°C will melt the gel, and after that, it can form gel again.

The turbidity test results presented in Table 2

showed that turbidity tests were below 1% both from centrifugation and heating treatment. According to Cho *et al.* (2008), a stable microemulsion had a turbidity value of <1%. The turbidity values of the samples after centrifugation treatment were not different from the heating treatment. The stability possessed by the sample was probably due to the different surfactant compositions. Cho *et al*. (2008) explained that the mixture of surfactants with different concentrations would form tiny particles that increased the microemulsion's solubility and stability.

3.1.2 Conductivity test

The electrical conductivity was used to determine the type of microemulsion formed. Conductivity could show differences in W/O (water in oil), bicontinuous, and O/W (oil in water) microemulsions based on the microemulsion microstructure formed (Suhendra *et al.*, 2012). The electrical conductivity of the A1-A10 sample, which had a water content of 0-90% was presented in Figure 1. All samples had a peak of conductivity level around 60-70% water content, in 175-200 µS/cm. This condition was presumably because the O/W microemulsion began to form. Cho *et al.* (2008) studied that microemulsion with canola oil had a high conductivity, namely 276.1 µS/cm, which indicated an oil-in-water phase.

All samples A, B, and C with water content of 0-40% had low conductivities. This condition was caused by the formation of the W/O phase. According to Constantinides and Scalart (1997), very low conductivity was a characteristic of water in oil (W/O) microemulsions, while Bumajdad and Eastoe (2004) explained that oil in water (O/W) microemulsions have relatively high conductivity. At 50-60% water content, the slope increased. At this stage, a bicontinuous phase was probably being formed. This was explained by Xu *et al.* (2016) that a microemulsion with a water content of 60% would form an O/W microemulsion that the conductivity value will be higher. The conductivity peak (Figure 1) was in the sample with a water content of 70% and then decreased with increasing water content. Xu *et al.* (2016) explained that increasing water content causes decreasing conductivity due to diluting with distilled water. At this stage, the microemulsion also had good mobility along with its low viscosity.

Based on the results of the conductivity test, samples A5-A9, B5-B9, and C5-C9, which had a water content of 10-50%, were not further tested because they were estimated to have W/O and bicontinuous phases, so they were not following the research objectives that focused on microemulsion O/W. Samples A4, B4, and C4, which had a water content of 60%, will also not be continued because they were too thick to apply to food products later. Fadilah (2010) proved that a microemulsion with a water content of 60% was too viscous/thick to affect the sensory quality of the product, although it had a high

Table 2. Turbidity and appearance of various surfactant and water mixtures after centrifugation (3000 rpm, 20 mins) and heating (105°C, 5 hours)

After centrifugation (3000 rpm, 20 mins)								
Formula A			Formula B			Formula C		
Code	Turbidity (%)	Appearance	Code	Turbidity (%)	Appearance	Code	Turbidity (%)	Appearance
A1	0.041[a]	Transparent	B1	0.035[a]	Transparent	C1	0.016[a]	Transparent
A2	0.062[a]	Transparent	B2	0.041[a]	Transparent	C2	0.018[a]	Transparent
A3	0.064[a]	Transparent	B3	0.053[a]	Transparent	C3	0.021[a]	Transparent
A4	0.094[a]	Transparent	B4	0.069[a]	Transparent	C4	0.030[a]	Transparent
A5	X	Gel	B5	X	Gel	C5	X	Gel
A6	0.117[b]	Transparent	B6	0.154[b]	Transparent	C6	0.055[a]	Transparent
A7	0.166[c]	Transparent	B7	0.168[b]	Transparent	C7	0.108[b]	Transparent
A8	0.196[c]	Transparent	B8	0.210[c]	Transparent	C8	0.143[c]	Transparent
A9	0.154[c]	Transparent	B9	0.223[c]	Transparent	C9	0.198[c]	Transparent
After heating at 105°C, 5 hours								
Formula A			Formula B			Formula C		
Code	Turbidity (%)	Appearance	Code	Turbidity (%)	Appearance	Code	Turbidity (%)	Appearance
A1	0.055[a]	Transparent	B1	0.051[a]	Transparent	C1	0.023[a]	Transparent
A2	0.074[ab]	Transparent	B2	0.064[a]	Transparent	C2	0.025[a]	Transparent
A3	0.081[ab]	Transparent	B3	0.067[a]	Transparent	C3	0.028[a]	Transparent
A4	0.067[a]	Transparent	B4	0.064[a]	Transparent	C4	0.028[a]	Transparent
A5*	X	Gel	B5*	X	Gel	C5*	X	Gel
A6	0.127[b]	Transparent	B6	0.366[c]	Transparent	C6	0.069[b]	Transparent
A7	0.177[c]	Transparent	B7	0.143[b]	Transparent	C7	0.101[c]	Transparent
A8	0.198[d]	Transparent	B8	0.187[c]	Transparent	C8	0.122[c]	Transparent
A9	0.164[c]	Transparent	B9	0.244[d]	Transparent	C9	0.120[c]	Transparent

Note: *forming a gel after cooling.

Values with different superscripts within the same column are significantly different ($p<0.05$). Source: Ningrum (2020).

conductivity. Fanun (2008) explained that the microemulsion's increased viscosity due to the phase shift from the monostructure to the bicontinuous structure. It means the shift of the O/W phase to the bicontinuous phase. The selected samples for further research were A1, A2, A3, B1, B2, B3, C1, C2, and C3, which had a water content of 70-90%.

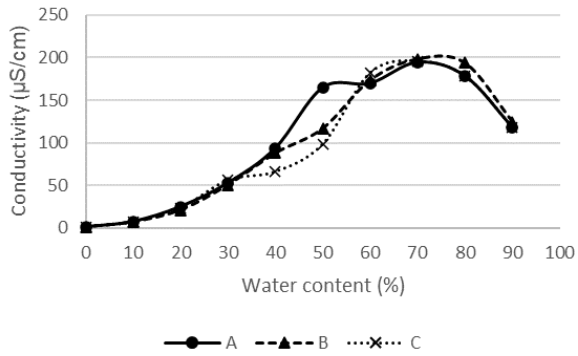

Figure 1. Conductivity of microemulsion. Source: Ningrum (2020)

3.2 O/W Microemulsion turbidity test

The samples that passed the first turbidity and conductivity test were A1, A2, A3, B1, B2, B3, C1, C2, and C3. Each sample was then added with various fatty acid concentration extracts of 0 ppm, 100 ppm, 200 ppm, and 300 ppm. The labelling samples of A1-0, A1-100, A1-200, and A1-300 meant A1 samples with 0, 100, 200, and 300 ppm *U. lactuca* fatty acid extract. The same labelling system was applied to samples A2, A3, B1, B2, B3, C1, C2, and C3. The sample with 0 ppm fatty acid extract served as a control. Samples were incubated for 24 hours, centrifugated and heating treatment, and then tested for turbidity. The turbidity test results are presented in Table 3.

The turbidity after centrifugation treatment of control samples A1-0, A2-0, B1-0, B2-0, B3-0, C1-0, C2 -0, and C3-0 had the smallest turbidity values than the non-control samples. ANOVA test results showed that the turbidity value was significantly different ($P<0.05$), it was later further tested. Further test results could be seen in Table 3. The samples that had the best stability were the samples that were not significantly different from the control samples and had the lowest turbidity in both treatments, namely samples A1-100, A2-100, A2-200, B1-100, B2-100, B3-100, B3-200, C1-100, C2-100, C2-200, and C3-100. Based on these results, the microemulsion of *U. lactuca* fatty acid extract was stable

Table 3. Turbidity index of microemulsion after centrifugation (3000 rpm, 20 mins) and heating (105°C, 5 hours).

After Centrifugation (3000 rpm, 20 mins)					
Sample	Turbidity (%)	Sample	Turbidity (%)	Sample	Turbidity (%)
A1-0	0.03132^{i}	B1-0	0.03869^{i}	C1-0	0.0479^{i}
A1-100	$0.3464^{bcdefghi}$	B1-100	$0.3399^{bcdefghi}$	C1-100	$0.407^{bcdefghi}$
A1-200	0.857^{a}	B1-200	0.5426^{abcde}	C1-200	0.6108^{abcde}
A1-300	0.6734^{abcde}	B1-300	0.7020^{abcd}	C1-300	0.5969^{abcde}
A2-0	0.034084^{i}	B2-0	0.03316^{i}	C2-0	0.03316^{i}
A2-100	$0.3003^{bcdefghi}$	B2-100	0.2460^{efghi}	C2-100	$0.3215^{bcdefghi}$
A2-200	$0.4588^{abcdefghi}$	B2-200	0.5693^{abcde}	C2-200	$0.40993^{bcdefghi}$
A2-300	0.6568^{abcde}	B2-300	0.5905^{abcde}	C2-300	0.617^{abcde}
A3-0	0.6762^{abcde}	B3-0	0.04238^{i}	C3-0	0.04238^{i}
A3-100	0.6375^{abcde}	B3-100	$0.3197^{bcdefghi}$	C3-100	$0.3851^{bcdefghi}$
A3-200	0.6015^{abcde}	B3-200	$0.4680^{abcdefghi}$	C3-200	$0.4984^{abcdefg}$
A3-300	$0.5048^{abcdefg}$	B30-300	0.7406^{ab}	C3-300	0.620^{abcde}
After Heating (105°C, 5 hours)					
Code	Turbidity (%)	Code	Turbidity (%)	Code	Turbidity (%)
A1-0	0.03408^{i}	B1-0	0.03869^{i}	C1-0	0.0590^{ghi}
A1-100	$0.2847^{cdefghi}$	B1-100	$0.2902^{cdefghi}$	C1-100	$0.341^{bcdefghi}$
A1-200	$0.3399^{bcdefghi}$	B1-200	$0.4597^{abcdefghi}$	C1-200	0.5196^{abcdef}
A1-300	0.568380^{abcde}	B1-300	0.6098^{abcde}	C1-300	0.5426^{abcde}
A2-0	0.04422^{i}	B2-0	0.04974^{i}	C2-0	0.04514^{i}
A2-100	$0.2773^{cdefghi}$	B2-100	0.2644^{defghi}	C2-100	$0.343^{bcdefghi}$
A2-200	$0.43481^{abcdefghi}$	B2-200	0.5380^{abcde}	C2-200	$0.4753^{abcdefghi}$
A2-300	0.6559^{abcde}	B2-300	0.5500^{abcde}	C2-300	0.667^{abcde}
A3-0	0.0746^{fghi}	B3-0	0.05896^{ghi}	C3-0	0.050666^{hi}
A3-100	$0.3353^{bcdefghi}$	B3-100	$0.3077^{bcdefghi}$	C3-100	$0.372^{bcdefghi}$
A3-200	0.5177^{abcdef}	B3-200	$0.4735^{abcdefghi}$	C3-200	$0.4965^{abcdefgh}$
A3-300	$0.4569^{abcdefghi}$	B3-300	0.7204^{abc}	C3-300	0.6983^{abcd}

Note: 0-300 (ppm) indicated the addition of *U. lactuca* fatty acid extract

Values with different superscripts within the same column are significantly different ($p<0.05$). Source: Ningrum (2020)

in the water content range of 70% to 90%, with 100 ppm to 200 ppm of fatty acid extract.

Another study was conducted by Suhendra *et al.* (2014), which produced a stable microemulsion formula with a water content of 65% with a surfactant ratio Tween 80: Span 80: Tween 20: 92: 5.5: 2.5 (% v/v), while Sary's research (2019) regarding the application of *U. lactuca* fatty acid extract microemulsion in lemon juice drinks, produced a stable microemulsion formula with a water content of 46% and a fatty acid concentration of 300 ppm. The difference in stable microemulsion formula with another study was due to differences in the materials used.

The microemulsion of 300 ppm *U. lactuca* fatty acid extract had a particle size of 12.6-14.6 nm (Sary, 2019). The particle size met Cho *et al.* (2008), microemulsion standards that had a range of 5–100 nm. Based on these results, microemulsions with smaller concentrations of *U. lactuca* fatty acid extract, namely 100 and 200 ppm were assumed to have smaller particle sizes so that they would not exceed the standard microemulsion particle size.

3.3 Peroxide value

The O/W microemulsion test has determined the samples with good stability, namely samples A11, A21, A22, B11, B21, B31, B32, C11, C21, C22, and C31. The samples were then tested for peroxide values. Peroxide compounds were products formed at the beginning of the fat oxidation process so that the peroxide levels in fat indicate the level of fat oxidation damage (Andarwulan *et al.*, 2011). The method used was ferritocyanate by Hills and Tiel modified by Chapman and Mackay in Adnan (1980). The results of the peroxide value test are presented in Table 4.

Table 4. Peroxide value of stable microemulsion

No.	Code	Peroxide value (mEq/kg)	No.	Code	Peroxide value (mEq/kg)
1	A1-0	2.01±0.89	11	B3-100	1.53±0.31
2	A1-100	1.79±0.15	12	B3-200	1.79±0.05
3	A2-0	1.94±0.89	13	C1-0	1.60±0.31
4	A2-100	1.94±0.75	14	C1-100	1.41±0.25
5	A2-200	2.03±0.46	15	C2-0	2.46±0.79
6	B1-0	1.45±0.05	16	C2-100	1.65±0.33
7	B1-100	1.77±0.41	17	C2-200	2.18±0.38
8	B2-0	1.65±0.49	18	C3-0	2.29±0.38
9	B2-100	1.60±0.14	19	C3-100	2.01±0.83
10	B3-0	1.70±0.52			

Source: Ningrum (2020)

In the Joint FAO/WHO Codex Alimentarius Commission (2015), the limit of the permissible rate of peroxide in fish oil was ≤5 mEq/kg. Based on these standards, all samples tested for peroxide value did not exceed the predetermined limits, as shown in Table 4. This condition indicated that the microemulsion formula in the tested sample protected the *U. lactuca* fatty acid content well from the oxidation process. The highest concentration of fatty acids added stably to the microemulsion formula was 200 ppm, with the lowest percentage of surfactant being 20%.

Another study was conducted by Suhendra *et al.* (2014) which produced a stable microemulsion formula with a water content of 65% with a surfactant ratio Tween 80: Span 80: Tween 20: 92: 5.5: 2.5 (% v/v). In comparison, the application of *U. lactuca* fatty acid extract microemulsion in lemon juice drinks produced a stable microemulsion formula with a water content of 46% and a fatty acid concentration of 300 ppm (Sary, 2019). The difference in the results regarding the stable microemulsion formula from this study with other studies could be due to differences in the ratio of the surfactant.

Surfactants often caused a soap-like taste and decrease the utilization in the food system (de Rovira, 2017). The soap-like taste was caused by the content of free fatty acids in the surfactants, namely lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic acids. Polysorbate 20 or Tween 20 and Polysorbate 80 or Tween 80 had almost all free fatty acids (Guler, 2005; Cadwallader *et al.,* 2007; Siska *et al.*, 2015). The amount of surfactant used will affect the cost of microemulsion production. The less surfactant used, the lower the production costs, but according to Suhendra *et al.* (2014), too low a surfactant concentration could cause decreasing in microemulsion stability. Critical Micelle Concentration (CMC) point, or the concentration at which surfactants dissolved in water, will gather spontaneously and form micelles (Georges and Chen. 1986). Surfactants below the CMC point would tend to be in monomers and had a high face tension, while microemulsions had a low face tension (Suhendra *et al.,* 2014).

The best sample based on the peroxide number test was the sample with the lowest surfactant content or concentration. Samples A2-200 (Tween 80: Span 80: Tween 20 = 92%: 5.5%: 2.5%) and C2-200 (Tween 80: Span 80: Tween 20 = 87%: 5.5%: 7.5%) had the lowest surfactant content (20%) and 80% water content and the highest fatty acid extract concentration, namely 200 ppm *U. lactuca f*atty acid extract.

4. Conclusion

The stable microemulsion was the microemulsion with surfactant concentration of 20% (Tween 80: Span 80: Tween 20 = 92: 5.5: 2.5 (% v/v) and Tween 80: Span 80: Tween 20 = 87: 5.5: 7.5 (% v/v)), with the addition of 80% water and 200 ppm fatty acids. The *U. lactuca* fatty acid microemulsion was stable at room temperature for 24 hrs, at 105°C for 5 hrs, and centrifugation at 3000 rpm for 20 mins.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Universitas Gadjah Mada for Final Project Recognition Program. letter of assignment number 2488/UN1.P.III/DIT-LIT/PT/2020. May 12th, 2020. This paper consists of data from the first author's undergraduate thesis.

References

Abbasi, S. and Radi, M. (2016). Food grade microemulsion systems: Canola oil/lecithin: n-propanol/water. *Food Chemistry*, 194, 972–979. https://doi.org/10.1016/j.foodchem.2015.08.078

Adnan, M. (1980). Lipid Properties and Stability of Partially Defatted Peanuts. USA: University of Illinois. Dissertation.

Andarwulan, N., Kusnandar, F. and Herawati, D. (2011). Analisis Pangan. Jakarta, Indonesia: Dian Rakyat. [In Bahasa Indonesia]

Bumajdad, Z. and Eastoe, J. (2004). Conductivity of water-in-oil microemulsions stabilized by mixed surfactants. *Journal of Colloid and Interface Science,* 274(1), 268–276. https://doi.org/10.1016/j.jcis.2003.12.050

Burguera, J.L. and Burguera, M. (2012). Analytical applications of emulsions and microemulsions. *Talanta, 96(1), 11–20. https://doi.org/10.1016/j.talanta.2012.01.030*

Cadwallader, K.R., Singh, T.K. and Jerrell, J.P. (2007). StreamLined analysis of short-. medium. and long-chain free fatty acids in dairy products. *Flavor of Dairy Products*, 111–122. https://doi.org/10.1021/bk-2007-0971.ch006

Cho, Y.H., Kim, S., Bae, E.K., Mok, C.K. and Park, J. (2008). Formulation of a cosurfactant-free *O/W* microemulsion using nonionic surfactant mixtures. *Journal of Food Science,* 73(3), E115–E121. https://doi.org/10.1111/j.1750-3841.2008.00688.x

Constantinides, P.P. and Scalart, J. (1997). Formulation and physical characterization of water-in-oil-microemulsions containing long-versus medium-chain glycerides. *International Journal of Pharmaceutics,* 158(1), 57–68. https://doi.org/10.1016/S0378-5173(97)00248-2

de Rovira, D. (2017). Dictionary of Flavors. 3rd ed. United Kingdom: John Wiley and Sons Ltd. https://doi.org/10.1002/9781118856420

Fadilah, R. (2010). Formulasi and Aplikasi Mikroemulsi Lutein untuk Menghambat Fotooksidasi Vitamin C dalam Sari Buah Apel. Universitas Gadjah Mada. Yogyakarta, Indonesias: Thesis. [In Bahasa Indonesia].

Fanun, M. (2008). A study of the properties of mixed nonionic surfactants microemulsions by NMR. SAXS. viscosity and conductivity. *Journal of Molecular Liquids,* 142(1-3), 103–110. https://doi.org/10.1016/j.molliq.2008.05.006

Flanagan, J. and Singh, H. (2006). Microemulsions: a potential delivery system for bioactive in food. *Critical Reviews in Food Science and Nutrition*, 46 (3), 221–237. https://doi.org/10.1080/10408690590956710

Georges, J. and Chen, J.W. (1986). Micellization Study of Sodium Dodecyl Sulfate in Water and Microemulsion Systems by Conductivity and Counterion-Activity Measurements. *Journal of Colloid and Interface Science*, *113(1), 143–153. https://doi.org/10.1016/0021-9797(86)90214-6*

Ginsberg, H.N. and Karmally, W. (2000). Nutrition. lipids. and cardiovascular disease. Biochemical and Physiological Aspects of Human Nutrition. Philadelphia, USA: WB Saunders Company.

Guler, Z. (2005). Quantification of free fatty acids and flavor characteristics of kasar cheeses. *Journal of Food Lipids,* 12(3), 209–221. https://doi.org/10.1111/j.1745-4522.2005.00018.x

Haering, G. and Luisi, P.L. (1986). Hydrocarbon gels from water-in-oil microemulsions. *Journal of Physical Chemistry*, 90, 5892–5895. https://doi.org/10.1021/j100280a086

Handayani, T. (2016). Karakteristik dan Aspek Biologi *Ulva* spp. (Chlorophyta. Ulvaceae). *Oseana*, 41(1), 1 –8 [In Bahasa Indonesia].

Joint FAO/WHO Codex Alimentarius Commission. (2015). Codex Alimentarius: Fats and Oils. Geneva: Food and Agriculture Organization of the United Nations.

Kumari, P., Reddy, C.R.K. and Jha, B. (2011). Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. *Journal of Analytical Biochemistry*, 415(2), 134–

144. https://doi.org/10.1016/j.ab.2011.04.010

Lawrence, M.J. and Rees, G.D. (2012). Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews*, 64(1), 175–193. https://doi.org/10.1016/j.addr.2012.09.018

Luisi, P.L., Scartazzini, R., Haering, G. and Schurtenberger, P. (1990). Organogels from water-in -oil microemulsions. *Colloid and Polymer Science*, 268(4), 356–374. https://doi.org/10.1007/ BF01411679

Ningrum, Y.P. (2020). Variasi Jenis dan Konsentrasi Surfaktan untuk Formulasi Mikroemulsi Asam lemak *Ulva lactuca*. Indonesia: Universitas Gadjah Mada, BSc. Thesis. [In Bahasa Indonesia].

Safia, R.N. (2013). Jenis dan Sebaran Makroalga di Zona Intertidal Pantai Ngandong dan Drini Kabupaten Gunungkidul. Indonesia: Fakultas Pertanian. Universitas Gadjah Mada. Thesis. [In Bahasa Indonesia].

Safitri, R. (2019). Formulasi Mikroemulsi Asam Lemak Makroalga *Ulva lactuca* serta Aplikasinya pada Minuman Jelly. Indonesia: Fakultas Pertanian. Universitas Gadjah Mada. Thesis. [In Bahasa Indonesia].

Sagiri, S.S., Behera, B., Sudheep, T. and Pal, K. (2012). Effect of composition on the properties of Tween-80 –Span-80-based organogels. *Designed Monomers and Polymers*, 15(3), 253–273. https:// doi.org/10.1163/156855511X615669

Saraswati, D. (2020). Pengaruh Volume. Kecepatan Pengadukan. dan Lama Ekstraksi Pada Produksi Asam Lemak *Ulva lactuca*. Indonesia: Fakultas Pertanian. Universitas Gadjah Mada. Thesis. [In Bahasa Indonesia].

Sary, F.M. (2019). Formulasi Mikroemulsi Asam Lemak Makroalga *Ulva lactuca* serta Aplikasinya pada Minuman Sari Buah Lemon. Indonesia: Fakultas Pertanian. Universitas Gadjah Mada. Thesis. [In Bahasa Indonesia].

Schmid, M., Guiheneuf, F. and Stengel, D.B. (2014). Fatty acid contents and profiles of 16 macroalgae collected from the Irish Coast at two seasons. *Journal of Applied Phycology,* 26, 451-463. https:// doi.org/10.1007/s10811-013-0132-2

Siska, C.C., Pierini, C.J., Lau, H.R., Latypov, R.F., Fesinmeyer, R.M. and Litowsk, J.R. (2015). Free Fatty Acid Particles in Protein Formulations Part 2: Contribution of Polysorbate Raw Material. *Journal of Pharmaceutical Sciences*, 104(2), 447–456. https://doi.org/10.1002/jps.24144

Suhendra, L., Hastuti P. and Hidayat, C. (2012). Formulasi dan Stabilitas Mikroemulsi O/W Sebagai Pembawa Fucoxanthin. *Agritech*, 32(3), 230-239. [In Bahasa Indonesia].

Suhendra, L., Raharjo, S., Hastuti, P. and Hidayat, C. (2014). Stabilitas Mikroemulsi Fucoxanthin dan Efektivitasnya dalam Menghambat Fotooksidasi Vitamin C pada Model Minuman. *Agritech*, 34(2), 138-145. [In Bahasa Indonesia].

Xu, Z., Jin, J., Zheng, M., Zheng, Y., Xu, X., Liu, Y. and Wang, X. (2016). Co-surfactant free microemulsions: Preparation. characterization and stability evaluation for food application. *Food Chemistry*, 204, 194–200. https://doi.org/10.1016/ j.foodchem.2016.01.073

Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C. and Attia, H. (2011). Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chemistry*, 128(4) 895–901. https://doi.org/10.1016/j.foodchem.2011.03.114

