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Research Paper

Okra Mucilage Extract as A Co-Surfactant Increased the Curcumin Nanoemulsion Stability and Encapsulation Efficiency

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Abstract
Curcumin has various bio-functional properties; however, curcumin poor bioavailability reduces its efficacy. Nanoemulsion delivery system is an alternative method improving curcumin bioavailability in which surfactant and oil used, play an important role in determining nanoemulsion properties. Several studies on curcumin nanoemulsions apply synthetic surfactants which can be harmful if they are added excessively. This study aims to use a natural emulsifying agent, namely okra mucilage extract (OME), and determine its effectiveness as co-surfactant. OME is safe to use as an emulsifying agent because it is natural, harmless, safe, biodegradable and eco-friendly. Liquid-liquid and microwave extraction methods were used to obtain OME which was further identified using Fourier Transfer Infrared Spectroscopy (FTIR). Meanwhile, sonication method was used to produce curcumin nano-emulsion (CurN). The particle size and polydispersity index of curcumin nano-emulsion were measured using Particle Size Analyzer (PSA) with Dynamic Light Scattering (DLS) technique, while the morphology of the nanoemulsion was observed using a Digital Imaging Microscope and Confocal Laser Scanning Microscope (CLSM). The results showed that the addition of 0.0160 g OME at a ratio of 1:5 (OME: Tween 80) in the preparation of 5 mL of CurN was able to reduce the particle size and polydispersity index from 740.80 ± 9.70 nm to 289.20 ± 2.23 and 0.340 ± 0.005 to 0.165 ± 0.008 respectively. OME increased the encapsulation efficiency from 77.93 ± 6.59% to 87.17 ± 1.12% which was confirmed by the augmentation of the fluorescence intensity of curcumin from 192.82 to 388.55. The addition of OME also maintained the stability of the CurN up to 14 days of storage at 4°C.

Keywords
Curcumin, Okra Mucilage Extract, Nanoemulsion

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1. INTRODUCTION

Turmeric (*Curcuma longa L.*) is one of the most common medicinal plants in Indonesia. Turmeric contains an active compound, curcumin, which has various bio-functional properties to prevent various diseases including diabetes. In addition, curcumin also has biofunctional properties such as anti-tumor, antioxidant, anti-inflammatory and anti-diabetic (Den Hartogh et al., 2019; Gabr et al., 2022; Jakubczyk et al., 2020; Kunnumakkara et al., 2017). However, curcumin has some disadvantages like poor bio-distribution, metabolism, and bioavailability. Based on several clinical trials, it is shown that curcumin is only detected in small amounts or even not being absorbed within cells (Dei Cas and Ghidoni, 2019; Md Saari et al., 2020). Curcumin is a lipophilic active substance and has poor polarity and dissolution rate in water which inhibits the distribution of curcumin to body organs (Toden and Goel, 2017). Thus, encapsulated curcumin in nanoemulsion system is being developed to increase the efficiency of curcumin as a drug (Kumar et al., 2016). Jannah, et al. developed curcumin nanoemulsion with a high encapsulation efficiency up to 81% in the system added with lecithin surfactant (Jannah et al., 2021). It also increased up to 90.56 ± 0.47% in the curcumin nanoemulsion system containing medium chain triglyceride (Sari et al., 2015).

Nanoemulsions are colloidal systems in the submicron size range that can act as carriers of drug molecules with an average size ranges from 10 to 1000 nm (Jaiswal et al., 2015). Nanoemulsions deliver bioactive compound to the target site which requires a stable system during its circulation in the body. The encapsulation and the release of bioactive compounds may be influenced by the type of emulsifiers, structural and compositional properties of the nanoemulsion systems (Chuacharoen et al., 2019). The main composition of nanoemulsion consists of bioactive substances, oil, water and surfactants. The type of oil and surfactant used are adjusted according to the type of bioactive substance to be encapsulated. Furthermore, the composition ratio is also a determining factor for the physiochemical properties and stability of the nanoemulsions (Chuacharoen et al., 2019). Pinheiro et al. (2013) compared the use of Tween 20, sodium dodecyl sulphate (SDS) and dodecyltrimethylammonium bromide (DTAB) surfactants in the manufacturing of curcumin nanoemulsions. The results show that different types of surfactants affect the stability and bioavailability of curcumin nanoemulsions in the body system (e.g. digestive and intestinal).

Several studies of curcumin nanoemulsion highlighted the use of a combination of synthetic and natural surfactants. Sari et al. (2015) developed curcumin nanoemulsions stabilized by Tween 80 (0.5-2%) and WPC-70 (0-1%), the most stable curcumin nanoemulsion was obtained with 2% Tween 80 and 0.5% WPC-70. Artiga-Artigas et al. (2018) compared the use of three types of surfactants, Tween 20, lecithin, and sucrose palmitate. The results showed that curcumin nanoemulsions containing Tween 20 showed the highest encapsulation efficiency values.

Nowadays, natural surfactants or emulsifying agents, which can be obtained from natural ingredients, such as hydrocolloids, have been developed. Hydrocolloids are components of long-chain polymers (including polysaccharides and proteins) that have gel-forming properties when dispersed in water. Hydrocolloids come from plants, animals, microbes, or components that generally contain hydroxyl groups. Based on their characteristics, hydrocolloids have been developed into emulsifying, thickening, and stabilizing agents (Herawati, 2018; Saha and Bhattacharya, 2010). Okra (*Abelmoschus esculentus L.*) extract, also known as a lady finger or gumbo, produces viscous mucilage in the form of hydrocolloid that contains polysaccharides and protein substances potential to be used as an emulsifier (Lousinian et al., 2017; Ritzoulis, 2017). Natural mucilage such as okra fruit mucilage extract tends to be safe to use as an emulsifying agent because it is natural, harmless, safe, biodegradable, and eco-friendly (Zaharuddin et al., 2014).

Noorlaila et al. (2015) studied the properties and characteristics of okra mucilage extract which is used as an emulsifier in coconut milk emulsion and showed that okra mucilage extract was a potential emulsifier. In addition, okra mucilage extract was also a potential drug delivery control agent. Pal (2020) encapsulated curcumin, thymoquinone, and piperine in a nanoparticle system using okra mucilage extract. The results showed that this system had a promising effect on inhibiting the growth of bacteria and cancer cells (Pal, 2020).

Apart from being used as an emulsifier, several studies have been conducted to find out the benefits of okra mucilage extract as an anti-bacterial, anti-diabetic, anti-cancer, and antioxidant (Alba et al., 2013; Dantas et al., 2021; Uddin Zim et al., 2021). The combination of two active substances, i.e. curcumin and okra mucilage extracts, is expected to give a synergistic effect as a herbal medicine. Thus, modifying curcumin nanoemulsion using okra mucilage extract as co-surfactant is expected to increase nanoemulsion stability and bioavailability as well as the anti-diabetic effect of the two active ingredients. This research aims to develop CurN system using OME as a co-surfactant in combination with Tween 80 at several concentrations. The observed characteristics of CurN were morphology, particle size, polydispersity index, encapsulation efficiency and stability.

2. EXPERIMENTAL SECTION

2.1 Materials and Tools

Materials used in this research are okra fruit, curcumin powder 95% (pharmaceutical grade, Health-Ingredients, China) which is used without further purification, Tween 80 (pharmaceutical grade J0203/20, PT Brataco, Indonesia), soybean oil (food grade, Mamasuka, Jinyuone Co., Ltd, Korea), methanol (A-1056, Pro Analysis, Smart Lab, Indonesia), acetone (technical grade), ethanol 96% (technical grade), and distilled water.

The tools used in this research are DelsaTM Nano C Particle Analyzer (Beckman Coulter, USA), Stereo Digital Camera Microscope (Olympus BX-51), Ultrasonic Cleaner Brand Skymen Cleaning Equipment (Shenzen Co Ltd 40 kHz), Confocal Laser Scanning Microscope (CSLM) Olympus FV1000, Magnetic Stirers ME HEI-TEC 145 (Heidolph Instruments GmbH & Co.KG, Germany), FTIR IRSpirit-T A22415801432 (Shimadzu Corporation), UV-Vis Spectrophotometer 1601 220 V (Shimadzu Corporation), Microwave 250W (Sharp, Indonesia), and ImageJ Software 1.44 version (Bethesda, Maryland, USA).

2.2 Methods

2.2.1 Okra Mucilage Extraction

Okra fruit was cut into small pieces and the seeds were removed from the fruit. Okra fruit was soaked in distilled water at 70°C for 4 hours with a ratio of okra fruit to distilled water of 1:5 followed with heating in microwave (250 W) for 2 minutes. OME was then separated using a filter cloth. Following the filtering, ethanol was added (using ratio of ethanol: extract 1:1) to isolate the mucilage which was then stored in the refrigerator for 5 days until a complete isolation was achieved. The precipitation as the result of isolation process was then filtered and rinsed with acetone. The precipitate obtained was dried in the oven at 60°C for 24 hours. The dried precipitate was stored in a desiccator and then characterized using FTIR instrument.

2.2.2 Curcumin Nanoemulsion Preparation

CurN with OME was prepared by mixing the aqueous phase and the oil phase. The aqueous phase was prepared by dissolving OME and Tween 80 in distilled water under continuous stirring for 24 hours at a speed of 200 rpm. The amount of OME and Tween 80 added were varied (Table 1). The oil phase was prepared by mixing 0.0250 g of curcumin with 200 μL of soybean-oil under continuous stirring for 2 hours at a speed of 200 rpm. The aqueous phase was added into the oil phase and then stirred for 15 minutes. Vials containing a mixture of the aqueous and oil phase were then sonicated using a sonicator bath with a frequency of 40 kHz for one 1 hour. For CurN without OME, the procedure applied was identical, nonetheless, there was no addition of OME in the aqueous phase.

2.2.3 Particle Size and Polydispersity Index Measurement

The particle size and polydispersity index of curcumin nanoemulsions were analyzed using DelsaTM Nano C Particle Analyzer based on Dynamic Light Scattering technique.

2.2.4 Curcumin Nanoemulsion Morphology

The morphology of the CurN were observed using a CLSM with a magnification of 400. Auto-fluorescence images were obtained using an excitation wavelength of 488 nm and an emission wavelength of 500 nm. Curcumin has fluorescence properties; this is due to the presence of two phenol groups which are conjugated with double bonds. Based on curcumin's fluorescence intensity we can predict curcumin concentration (Karimi et al., 2020).

2.2.5 Encapsulation Efficiency Determination

Concentration of curcumin encapsulated was measured based on the curcumin absorbance using UV-Vis spectrophotometer at 420 nm wavelength. Sample was diluted followed with centrifugation at 3500 rpm for 45 minutes. Then, 5 mL of the filtrate was taken and used for subsequent analysis to determine the amount of free curcumin using the UV-Vis method. The remaining filtrate was then diluted with methanol in a ratio of 1:1 and sonicated in a sonicator bath for 5 minutes to break the nanoemulsion system. Free curcumin and total curcumin were measured for their absorbance using a UV-Vis spectrophotometer. The amount of curcumin is calculated based on the absorbance value. Encapsulation efficiency was then determined using the following formula.

$$\%\text{EE} = \frac{\text{Total curcumin-Free curcumin}}{\text{Total curcumin}} \times 100\% \qquad (1)$$

2.2.6 Stability of Curcumin Nanoemulsion

The morphology of the CurN were observed by storing the samples at 4°C for 14 days. The samples were then observed using a Digital Imaging Microscope on day 7 and 14 to see morphological changes caused by destabilization of the nanoemulsions. The photos obtained from the microscope were analyzed using ImageJ software to predict the average particle size and particle size uniformity based on the calculated standard deviation.

3. RESULTS AND DISCUSSION

3.1 Okra Mucilage Extract

The resultant OME was in the form of thin reddish-brown flakes and the yield value was 0.9%. According to Lim et al. (2015), red color indicates the presence of tannin compounds. The OME was then identified using FTIR instrument to characterize functional groups. FTIR spectra (Figure 1) displayed an absorption at a wavelength of 3369 cm^{-1} which indicated the presence of an O-H group as the main functional group of the OME. The O-H group represents the hydrophilic characteristics in the polysaccharide while the moderate peak at 2935 cm^{-1} indicates the presence of C-H stretch in galactose and rhamnose. A small peak at 1719 cm^{-1} indicates the presence of C=O stretch which is a constituent of galacturonic acid. Then the medium peak of 1409 cm^{-1} indicates the presence of bending vibration O-H bonds which are constituents of galacturonic acid. Peak at 1200-1000 cm^{-1} indicates the C-O group contained in the aromatic compounds of galactose, rhamnose, and galacturonic acid. There are also amino acid functional groups identified, indicated by the presence of N-H group overlap with O-H at 3369 cm^{-1} and a C-N stretch group at a sharp peak at 1068 cm^{-1}. The identified functional groups signify that OME was also composed of hydrophilic and hydrophobic amino acids which contributed to the properties of OME as an emulsifying agent (Arjunan, 2021; Lim et al., 2015). Compared to the result of Lim et al. (2015) shown in Table 2, our results are similar which substantiates that the extraction process yielded the targeted compounds.

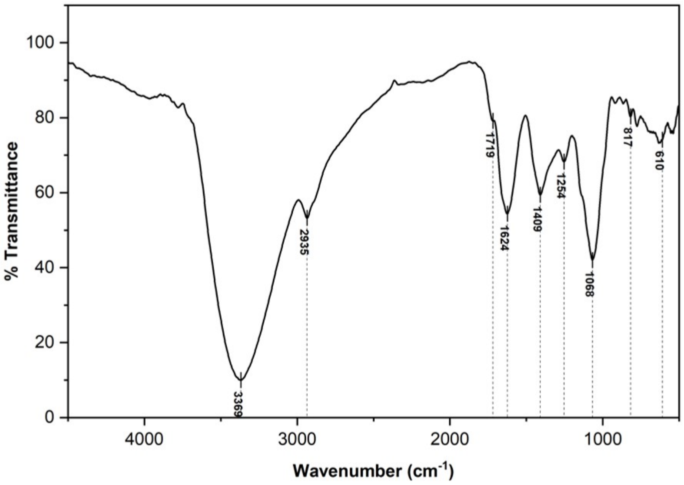

Figure 1. FTIR Spectra of OME from Our Research

3.2 Particle Size and Polydispersity Index

Particle size is one of the important parameters in drug delivery systems. Particle size affects the drug dissolution rate where this dissolution rate depends on the surface area of the interface (Sandri et al., 2014). The measurement of nanoemulsion particle size show some changes along with the addition of OME (Figure 2). Particle size either decreased or increased at a certain concentration of OME. CurN-0 has a particle size of 740.80 nm. The addition of OME as a co-surfactant as much as 0.0160 g (CurN-1); 0.0250 g (CurN-2) and 0.0500 g (CurN-3) in the preparation of 5 mL of CurN reduced particle size to 289.20 nm; 392.70 nm and 547.80 nm respectively. However, further addition of OME significantly increased the particle size to 1430.5 nm (CurN-4) without further augmentation in the CurN-5 (1338.1 nm). These results indicated that OME was able to reduce the particle size at a certain con-

Table 1. Composition of Curcumin Nanoemulsions

Sample Code	Curcumin (g)	Soybean Oil (µL)	Okra Mucilage Extract (g)	Tween 80 (g)	Aquadest (mL)
CurN-0	0.0250	200	0.0000	0.1000	4.7000
CurN-1 (1:5)	0.0250	200	0.0167	0.0835	4.7000
CurN-2 (1:3)	0.0250	200	0.0250	0.0750	4.7000
CurN-3 (1:1)	0.0250	200	0.0500	0.0500	4.7000
CurN-4 (3:1)	0.0250	200	0.0750	0.0250	4.7000
CurN-5 (5:1)	0.0250	200	0.0750	0.0167	4.7000

Table 2. Comparison of the FTIR Functional Groups

Functional Group	Wavenumber (cm^{-1})	
	Based on the Research	The Result of Lim et al. (2015)
O-H and N-H	3369	3413.3
C-H Stretch	2935	2925.8
C=O Stretch	1719	1722.3
O-H Bend	1409	1409.9
C-N Stretch	1068	1072.3

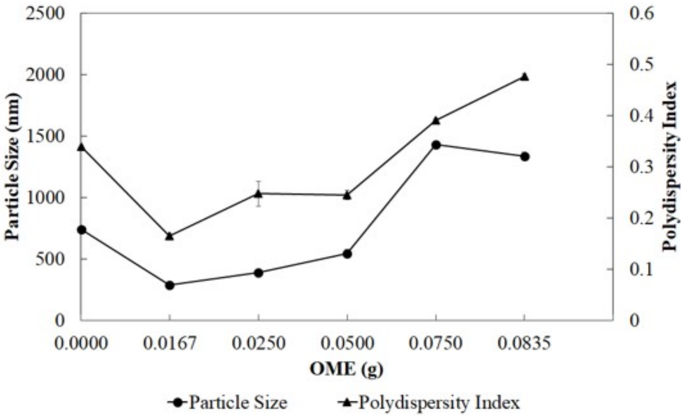

Figure 2. Particle Size and Polydispersity Index of CurN with Different Mass of OME Addition

centration. Further addition of OME affected inversely to the particle size; it was enlarged.

Similar to the particle size, the polydispersity index measurement also exhibited some changes as OME was added in the nanoemulsion system. Polydispersity index, with the value varies from 0.1 to 1, describes the degree of uniformity of the particle size distribution. A value of less than 0.1 indicates that the particle has good size uniformity in colloidal suspensions while a value close to 1 signifies that the particle size uniformity decreases (Subositi and Wahyono, 2019). All of our samples had polydispersity index values less than 0.5 which indicated that our system have relatively homogenous particle size distribution (Evan et al., 2011). Figure 2 shows the changes of polydispersity index in each OME concentration. With no OME addition, the polydispersity index of the nanoemulsion was 0.340. The index went down to 0.165; 0.247; and 0.245 when the nanoemulsions were prepared with 0.0167; 0.0250 and 0.0500 g of OME, a small polydispersity index indicates that the nanoemulsion has better size uniformity. On the contrary, the polydispersity index increased to 0.391 and 0.476 at OME addition of 0.0750 to 0.0835 g, respectively. Both indices exceeded that of nanoemulsion without OME addition, which implied the decrease of particle size uniformity.

From particle size and polydispersity index measurement, our results confirmed that the effect of OME could be divided into 2 regions. In the first region, OME reduced particle size as well as increased the system uniformity. On the other hand, in second region, OME increased particle size and reduced system uniformity.

3.3 Curcumin Nanoemulsion Morphology

Observation of the morphology of the CurN was carried out using a CLSM with a magnification of 400. Curcumin was shown as the bright green color in the image (Figure 3). Based on the 3D visualization, droplets of emulsion in a spherical shape were formed in CurN-0 dan CurN-1 systems, nonetheless, curcumin was more concentrated inside the droplet of the sample with added OME. The observed intensity in sample without OME as co-surfactant was 192.82 au the intensity of sample with added OME increased to 388.55 au. This signifies that OME enhanced the encapsulation of curcumin in the droplets and affected the morphology of the droplets.

3.4 Encapsulation Efficiency of Curcumin Nanoemulsions

Encapsulation efficiency represents the amount of curcumin trapped in the emulsion system. Encapsulation efficiency is an important parameter to consider when evaluating the success of a nanoemulsion in drug delivery system (Hudiyanti et al.,

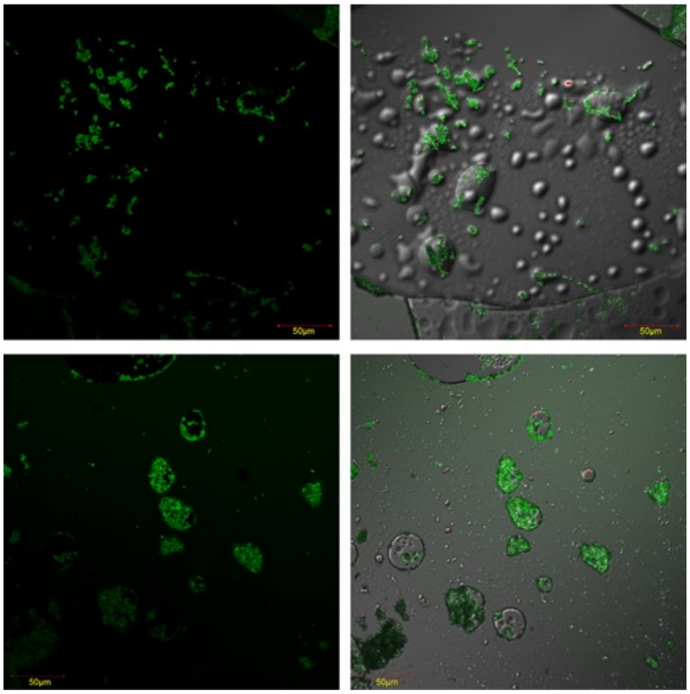

Figure 3. The CLSM Images of CurN-0 (above) and CurN-1 (below). CurN-0 is a CurN without the Addition of OME and CurN-1 is a CurN with the Addition of OME Which has the Smallest Particle Size. The Brightness in the Images was Caused by the Auto-fluorescence of Curcumin (488 nm Excitation). Note That the Dimension of the Droplets was Larger for the Sake of Image Clarity. The Scale Bar is Equal to 50 μm

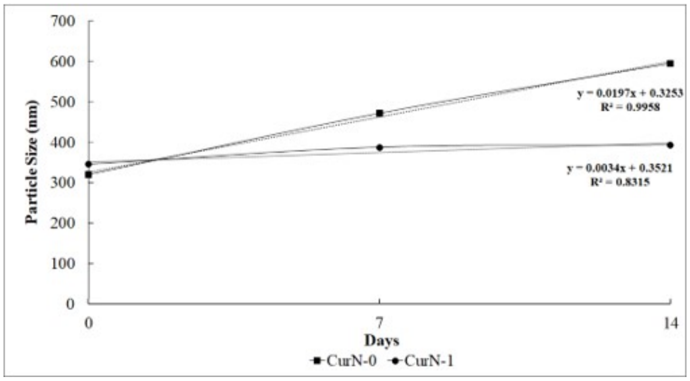

Figure 4. Predicted Average Particle Size as a Function of Storage Time of CurN-0 and CurN-1. CurN-0 is a CurN without the Addition of OME and CurN-1 is a CurN with the Addition of OME Which Has the Smallest Particle Size

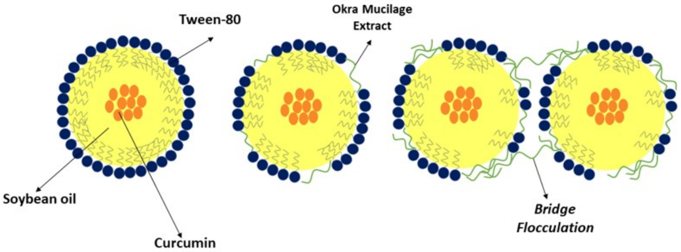

Figure 5. Illustration Scheme of CurN Based on the Research Results

2022). In this study, the encapsulation efficiency of CurN is measured on CurN-0 and CurN-1 (Table 3).

Table 3 shows that the addition of okra mucilage extract as co-surfactant increases the encapsulation efficiency of curcumin. The use of both Tween 80 and OME increased encapsulation efficiency by 10%. This augmentation was confirmed by the increase of curcumin intensity inside the droplets as shown in Figure 3 From these results it can be seen that the combination of two types of surfactants could increase the amount of curcumin trapped in the nanoemulsion system (Marin et al., 2016).

3.5 Stability of Curcumin Nanoemulsions

In this study, stability observations were made on nanoemulsions stored at 4°C on day 7 and 14 (Chuacharoen et al., 2019). Stability is determined from the changes in particle size and its diversity. Unstable system shows a drastic change of particle size and uniformity during storage due to particle coalescence to form larger and irregular particle size (Zhang and McClements, 2018). Figure 4 shows the increase of predicted average particle size as a function of storage time both in the system with and without OME. Linear regression was applied to determine the slopes of the curves which indicated the rate of particle size increase. The higher the value of the slope, the particle size increases faster which signifies the instability of the system. Based on the data obtained in Figure 4, it can be seen that the slope of CurN system without OME was three times higher than that of the system with added OME. This tells us that OME increased system stability.

Fundamentally, nanoemulsion systems are thermodynamically unstable. This is due to the lower free energy required to separate the oil and water phases when compared to the energy for the emulsification process, thus, nanoemulsion stability can change during certain storage time due to various mechanisms (Aswathanarayan and Vittal, 2019). Temperature can affect the stability of nanoemulsions by changing the physical properties in the oil and water phases of the emulsion systems. At high temperatures, the viscosity of the nanoemulsions decrease and cause damage to the interface layer and it can cause coalescence (Harun et al., 2018; Liu et al., 2019).

OME had the potential ability to stabilize the drug delivery system, increasing the encapsulation of active compounds in the drug delivery system (Harun et al., 2018). Polysaccharides can adsorb at the oil-water interface and reduce the interfacial tension, thus, able to form emulsion droplets that are more stable in protecting the lipophilic active substances (Shao et al., 2020). An illustrative schematic of the nanoemulsion from

Table 3. The Encapsulation Efficiency of Curcumin Nanoemulsions

Sample	Curcumin (g)	Okra Mucilage Extract (g)	Tween 80 (g)	%Encapsulation Efficiency
CurN-0	0.0250	0.0000	0.1000	77.93 ± 6.59*%
CurN-1	0.0250	0.0167	0.0835	87.17 ± 1.12*%

*Standard deviation value of three data

the research results is exhibited in Figure 5. In the figure, the hydrophobic group of tween-80 binds to oil and its hydrophilic group binds to water so that it can form a nanoemulsion system that encapsulates curcumin. We believe with the addition of OME, which is adsorbed onto the surface of the oil and water droplets, this OME together with tween-80 might increase and maintain the stability of the nanoemulsion by lowering the oil-water surface tension and increase the viscosity of the nanoemulsion so that it can prevent droplet coalescence. Whereas the increasing particle size and decreasing system uniformity could be due to the binding of OME molecules, which are situated at the surface of the droplets, with each other to form a bridge that facilitates the incorporation of particles (flocculation) as shown in the illustration on the right in Figure 5. Hence, it increased the particle size and made the nanoemulsion unstable (Shao et al., 2020).

4. CONCLUSION

OME in our study can reduce the particle size and polydispersity index of CurN at certain concentrations. At our best observation, the use of 0.016 g of OME (at OME to Tween 80 ratio of 1:5) in the preparation of 5 mL of CurN decreased the particle size and polydispersity index of curcumin nanoemulsion from 740.80 to 289.20 nm, and the polydispersity index value from 0.340 to 0.165. The addition of OME increased the fluorescence intensity of trapped curcumin from 192.82 to 388.55 au. This result was confirmed by an increase of encapsulation efficiency from 77.93 ± 6.59 to 87.17 ± 1.12% as observed using UV-Vis spectrophotometer. Furthermore, OME maintained the stability of the CurN for up to 14 days at 4°C. Natural emulsifiers, e.g. okra mucilage extract, can be used as an option to reduce the use of synthetic surfactants and to improve the quality of nanoemulsion in drug delivery systems.

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