



## PICKERING EMULSION PROPERTIES GENERATED BY NANOFIBRILLATED CELLULOSE ISOLATED FROM OIL PALM FRUIT BUNCH (OPEFB) AS A STABILIZER

Ira Desri Rahmi<sup>1,2</sup>, Erliza Hambali<sup>\*1</sup>, Farah Fahma<sup>1</sup>, Dwi Setyaningsih<sup>1</sup>

<sup>1</sup>Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, IPB University, Dramaga, Bogor, Indonesia

<sup>2</sup>Department of Agroindustrial Technology, Faculty of Agricultural Technology, Andalas University, Padang, Indonesia

\*Corresponding author

Email: [erlizahambali@apps.ipb.ac.id](mailto:erlizahambali@apps.ipb.ac.id)\*

**Abstract.** *This study aims to find the optimal nanofibrillated cellulose (NFC) concentration isolated from oil palm empty fruit bunch (OPEFB) particles to form stable pickering emulsions against creaming and coalescence. The emulsification process is based on a combination of homogenizer and ultrasonication. Pickering emulsion was prepared by mixing the dispersed phase (palm oil) and the dispersing phase (NFC concentration of 0.05 - 0.7 w/v%) at the ratio of 10:90. Fresh emulsion has a milky white appearance and is homogeneous. However, some samples' creaming process occurred on the 30<sup>th</sup> day of storage. Microscopic observations show that the droplets are round with various sizes. Differences in NFC concentrations significantly affect droplet size, zeta potential, rheology, and emulsion stability. Increasing the NCF concentration resulted in smaller droplet sizes, viscosity, zeta potential, and pickering emulsion stability. The emulsion has high stability against coalescence and creaming. NFC at 0.7 w/v% generates an emulsion with the best characteristics and high stability against creaming and coalescence. OPEFB-based NFC has the potential as a pickering emulsion stabilizer particle that can be applied to the food and non-food industries.*

**Keywords:** *Nanofiber cellulose; OPEFB; Pickering emulsion; Stabilizer.*

### 1. Introduction

Emulsion systems can generally be stabilized with surfactants or amphiphilic polymers. In the last decades, there has been a tendency to reduce the use of surfactants in a product formulation (less surfactant). This strategy can be achieved by applying solid particles as emulsion stabilizers [1,2,3]. "Pickering emulsions" are known as emulsions stabilized by solid particles, in which solid particles will be adsorbed at the (o-w) phase interface to produce steric protection and inhibit the coalescence (aggregation) of droplets [4]. Some inorganic nanoparticles have been studied as emulsion stabilizer particles, for example, silica, TiO<sub>2</sub>, CuO, and laponite [5]. However, accumulating these particles raises concerns due to their toxicity effects. Recently, organic particles (such as modified starch, nanocrystalline chitin, and nanocellulose) have received

attention as emulsion stabilizers due to their renewable nature, biodegradability, low toxicity, and biocompatible [6,7].

Cellulose dan its derivatives are promising renewable materials as emulsion stabilizers because they can be isolated from lignocellulosic based on agricultural biomass, which is abundant in nature dan low-cost. Oil palm empty fruit bunch (OPEFB) is one of the by-products of processing fresh fruit bunches of oil palm, with a high cellulose content of 44.2% [8]. The isolation process will affect the number of inter- and intra-molecular hydrogen bonds, crystallinity, and cellulose chain length [9]. Cellulose from OPEFB can be separated into nanocellulose, which has at least 1 dimension on the nanoscale (1-100 nm) consisting of nanofibrillated cellulose (NFC) and nanocrystalline cellulose (NCC) [7-8]. Compared to NCC, the NFC isolation process is carried out mechanically without chemicals such as sulfuric acid, which is vital for future sustainability. NFC has crystalline and amorphous parts with long, flexible, and entangled fibers [12]. Due to its high surface area to volume ratio, NFC has superior physicochemical properties, such as self-assembly at the interface [13]. Its unique properties and semi-flexible NFC promise a candidate for sustainable emulsion stabilizer particles [11-12].

Forming a pickering emulsion involves mixing the dispersed and dispersing phases and adding NFC particles as an emulsion stabilizer. The concentration of NFC particles in the dispersing phase is an essential factor influencing the formation of pickering emulsions. Several researchers found that the small droplet size in the pickering emulsion was proportional to the increase in the concentration of NFC particles [1, 4, 15]. Increasing the particle concentration increases the number of particles adsorbed at the o-w interface, which helps stabilize the interface area and limit the aggregation process [16]. When the particle concentration exceeds the critical loading concentration, the excess particles form a 3D network in the dispersing phase [17]. This effect increases the viscosity of the emulsion, thereby limiting the movement of the droplet and increasing the emulsion's stability. The stabilizer particles' correct concentration will form tiny droplets with an even distribution to obtain a stable pickering emulsion [18, 19].

Several researchers have used nanocellulose from oil palm empty fruit bunces (OPEFB) as an emulsion stabilizer .The utilization of NFC-OPEFB as an emulsion stabilizer has been carried out by Li *et al.* [20]. The NFC was obtained through a pretreatment process with Tempo oxidation and followed by high pressure homogenization. Stabilized Pickering emulsions were obtained at 2% CNF dosage with the emulsion stable against creaming and coalescence. Further, Ajayi *et al.* [19] used acid chloride (benzoyl chloride) to hydrophobically modify cellulose from OPEFB. The results showed that the Pickering emulsions stabilized by hydrophobic modification with benzoyl chloride were stable to coalescence. Nanocrystal cellulose (CNC) from palm press fiber also used

as pickering particle. CNC was obtained through combination of acid hydrolysis and high-pressure homogenization [21]. Pickering emulsion with 0,3% dosage of CNC exhibits large droplet but having a higher stability.

Although nanocellulose from OPEFB as an emulsion stabilizer has been reported, CNF produced by mechanical treatment (a combination of ultrafine grinding and ultrasonication) and without modification has never been reported before. This study aims to find the best concentration of NFC-based OPEFB particles to form pickering emulsions that are stable against creaming and coalescence processes. It provides information about using NFC from OPEFB as a stabilizer particle for pickering emulsions. The characteristics of a stabilizer-based pickering emulsion from NFC-based OPEFB can be used as basic information for product development in the pharmaceutical, biomedical, food, hygiene, and cosmetic industries.

## 2. Methods

### 2.1. Material

OPEFB is supplied from PTPN VI West Pasaman, West Sumatra, Indonesia. Sodium hydroxide and hydrogen peroxide are used to purify OPEFB cellulose. Meanwhile, palm oil is used for emulsion preparation

### 2.2. Cellulose and nanofibrillated cellulose isolation

The process of isolating cellulose and NFC-based OPEFB refers to a method modification by Fahma *et al.* [5,7]. OPEFB measuring  $\pm 0.5$ -1 cm was carried out by washing, boiling, and drying. Next, the fibers were delignified using NaOH and a bleaching process using H<sub>2</sub>O<sub>2</sub> to separate white cellulose fibers. Then, the cellulose was diluted to 1% (w/v) and suspended with a waring blender. NFC isolation was carried out using an ultra-fine grinder with a speed of 1500 rpm gap level -10 (5 processes), gap level -30 (5 processes), and gap level -50 (5 processes) in stages. Next, the suspension was ultrasonicated using an ultrasonic processor (Sonic Vibracell VCX 750) with a 1-inch diameter titanium alloy tip for 60 minutes at an amplitude of 80% and 20 kHz. NFC with 1% (w/v) is stored in the refrigerator.

### 2.3. Preparation of nanofibrillated cellulose stabilized pickering emulsion

The pickering emulsion was processed according to a modified study by Martins *et al.* [14] and Yuan *et al.* [22]. The ratio of the dispersed and dispersing phases is formulated at a constant (10:90). In preparing the dispersed phase, palm oil was heated to 45 °C for  $\pm 5$  minutes with 100 rpm stirring. Meanwhile, the dispersing phase consisting of NFC was prepared with 0.05%, 0.1%, 0.2%, 0.3%, 0.5%, and 0.7% (w/v) from dilution of 1% (w/v) NFC suspension. The dispersed phase was stirred using a homogenizer for  $\pm 2$  minutes and 6000 rpm. Next, the dispersing phase

was heated using a hotplate stirrer at 40°C, stirring at 600 rpm for 30 minutes. The oil is dripped gradually while stirring. Then, the mixture was homogenized at 9500 rpm using a T25 ultra turrax homogenizer for 1 minute. This process is followed by ultrasonication using a VCX-750 ultrasonic processor for 2 minutes (5 seconds on/ 3 of off), and every 30 seconds, the tool is turned off, and the sample is stirred manually. The sonication process uses a 1-inch probe, a frequency of 20 kHz, and an amplitude of 50%.

## 2.4. Characterization of pickering emulsions

### 2.4.1 Droplet size and distribution

The size and droplet distribution of the pickering emulsion was analyzed using a diffraction laser (Malvern Mastersizer 3000, Malvern Panalytical Ltd., Malvern, UK). The refractive index used is 1.33 (water phase) and 1.46 (oil phase). The stirring speed was set to 1800 rpm. The emulsion sample was added gradually using a pipette into the wet sample dispersion unit (Hydro EV) containing 250 ml of deionized water until an obscuration value of around 5-10% was obtained. This measurement produces  $d_{43}$  values,  $D_{10}$ ,  $D_{50}$ , and  $D_{90}$  values, Span factors, and uniformity values.

Meanwhile, the emulsion diameter is expressed in volume-weighted mean diameter ( $d_{43}$ ). The  $d_{43}$  value or de Brouckere mean diameter (de Brouckere mean diameter) is the spherical particles' average diameter with the same particle mass/volume ratio value. The  $d_{43}$  value is calculated based on Equation 1.

$$d_{43} = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \quad (1)$$

$D_i$  is the droplet diameter, and  $n_i$  is the number of droplets in the diameter  $d_i$ .

The span factor is a parameter that indicates the droplet size distribution uniformity, which is determined based on Equation 2.

$$\text{Span} = \frac{D_{v90} - D_{v10}}{D_{v50}} \quad (2)$$

$D_{v10}$ ,  $D_{v50}$ , and  $D_{v90}$  are diameters representing 10%, 50%, and 90% of the droplet size in the entire emulsion volume, with diameters smaller or equal to these values.

### 2.4.2. Zeta Potential

The electrophoretic droplet mobility as a  $\zeta$ -potential was measured using the electrophoretic light scattering (ELS) technique using a Litesizer 500 (Anton Paar GmbH, Austria). This measurement determines the surface electric charge of droplets dispersed in the continuous phase. Emulsions with high turbidity (turbidity) are diluted to a concentration of 0.01% (w/w) using distilled water to prevent some scattering effects.

### 2.4.3. Rheology

The pickering emulsion rheology was measured using a rheometer (Anton Paar MCR301, Anton Paar GmbH, Austria). The 5 ml sample was placed in a cone and plate measuring system (diameter = 50 mm and angle = 2°). The solution was first set at room temperature. Measurements were made in the Shear Rate range from 0.1 s<sup>-1</sup> to 1000 s<sup>-1</sup>. Flow behavior is measured based on shear stress (Pa), shear rate (s<sup>-1</sup>), and viscosity. The power law model (Equation 3) is used to quantify the emulsion flow behavior.

$$\sigma = k\dot{\gamma}^n \quad (3)$$

$\sigma$  is the Shear Stress (Pa),  $k$  is the consistency coefficient (Pa.sn),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>), and  $n$  is the power law index. The power law index shows the flow behavior. The value of  $n=1$  shows the flow behavior in the form of Newtonian. If  $n<1$ , then the solution behaves as a shear-thinning solution (pseudoplastic), and if  $n>1$ , then the solution behaves as a shear-thickening solution (dilatant).

### 2.4.4. Microscopic observation

The pickering emulsion droplets microstructure was observed with an optical microscope CX43 (Olympus, Japan). The pickering emulsion without dilution was placed on a glass slide and then slowly covered with a cover slip. The o-w emulsion microstructure images were taken utilizing a digital camera at 200x magnification.

### 2.4.5. Creaming index (CI)

The height of the emulsion is divided into three parts, namely the total height ( $H_t$ ), the upper phase (serum phase) height ( $H_s$ ), and the emulsified phase height ( $H_e$ ). The creaming index (CI) is measured based on the ratio of serum height and total emulsion height, as in Equation 4.

$$\text{Creaming index (CI) (\%)} = (H_s/H_t) \times 100\% \quad (4)$$

## 3. Results and Discussion

This study isolates NFC from OPEFB mechanically using an ultrafine grinder followed by an ultrasonication process. In the grinding stage with an ultrafine grinder, the cellulose fiber is reduced in size by three cycles, with each grinding 5 times in stages. Micrometer-sized cellulose was followed by an ultrasonication process for 60 minutes to produce NFC measuring  $54.80 \pm 0.85$  nm. Wu and Ma stated that the stabilizer particle size affects the formation and pickering emulsion stability [23]. Smaller particles have faster adsorption kinetics and have more efficient packing at the o-w interface than larger particles.

### 3.1. Visual appearance

Visually, the milky white pickering emulsion appeared to have only one layer when placed at room temperature for 1 day (Figure 1). This effect indicates that the oil droplet has been covered by NFC, as found by Liu *et al.* [24]. and Martins *et al.* [14]. The milky white layer is the emulsified phase of the emulsion system [25]. However, the creaming process in some samples was formed on the 30<sup>th</sup> day of storage. The creaming process is a separation caused by the dispersed phase density being smaller than the dispersing phase density and influenced by gravity [26]. Creaming was seen in the samples treated with 0.05%, 0.1%, and 0.2% pickering emulsion (marked with blue lines). The thick creaming layer was seen in the 0.05% and 0.1% pickering emulsion samples, and the thin creaming layer was seen in the 0.2% and 0.3% pickering emulsion samples. The 0.5% and 0.7% pickering emulsion treatments showed no creaming after storage in 30 days.

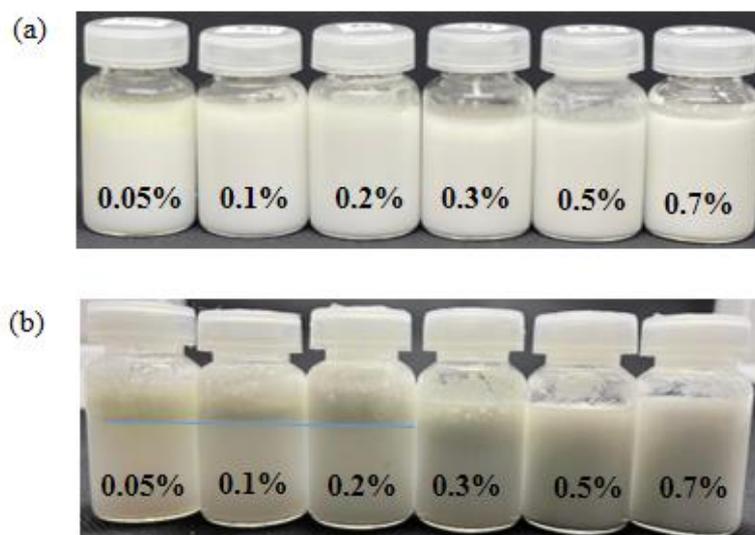


Figure 1. The pickering emulsion visual appearance at room temperature storage, a) Day 1, and b) Day 30.

### 3.2. Droplet size and distribution

Pickering emulsion droplets reported as  $d_{43}$  (volume-weighted mean diameter) are more sensitive to large droplets [26]. Generally, the droplet size of a pickering emulsion is a micrometer in scale ( $>1 \mu\text{m}$ ), which is categorized as a macroemulsion [27]. Micrometer-scale droplets can also be associated with the scattering effect of visible light (400-700 nm) so that the pickering emulsion has a milky white color, as shown in Figure 1. Analysis of variance showed that differences in NFC concentration had a dramatic effect on droplet diameter ( $d_{43}$ ), droplet size distribution (span index),  $D_{v10}$ ,  $D_{v50}$ , and  $D_{v90}$  values (Table 1).

This study produced droplet sizes ranging from  $9.19 \pm 0.01 \mu\text{m}$  to  $28.37 \pm 0.15 \mu\text{m}$ . In this case, the droplet size decreased with increasing the NFC concentration. At low concentrations of NFC (0.05 – 0.2%), the emulsion droplet size tends to be larger, namely  $>23.13 \pm 0.95 \mu\text{m}$ . The

droplet size significantly decreased when NFC was added with a concentration of 0.5%, which was around  $10.59 \pm 0.11 \mu\text{m}$ . This effect is related to the particles adsorbed on the droplet's surface in the dispersed phase, affecting emulsion stability. At a low NFC concentration, the number of adsorbed NFC particles is insufficient to cover the droplet surface, so the emulsion has larger droplets [20, 28]. Conversely, the amount of adsorbed NFC is sufficient to cover the emulsion droplet surface at a high NFC concentration, so the droplet will be tiny. Small droplet sizes are more helpful in stabilizing pickering emulsions [4]. The particle in more elevated the concentration, the more particles are adsorbed at the o-w interface, stabilizing the interface area and limiting the aggregation process. When the particle concentration exceeds the critical loading concentration, the excess particles will form a 3D network that will inhibit the droplet's movement, making the emulsion more stable [17]. This study found that the critical concentration of NFC loading to stabilize the emulsion was around 0.5%, characterized by the absence of a creaming process.

Table 1. Effect of NFC concentration on droplet diameter ( $d_{43}$ ), droplet size distribution (span index),  $D_{v10}$ ,  $D_{v50}$ ,  $D_{v90}$  values and uniformity index

Pickering Emulsions	$d_{4,3}$ ( $\mu\text{m}$ )	$D_{v10}$ ( $\mu\text{m}$ )	$D_{v50}$ ( $\mu\text{m}$ )	$D_{v90}$ ( $\mu\text{m}$ )	Span Index
Day 1					
0.05%	$28.37 \pm 0.15^f$	$1.43 \pm 0.01^a$	$10.35 \pm 0.57^c$	$74.47 \pm 0.38^f$	$7.08 \pm 0.4^f$
0.1%	$25.57 \pm 0.21^e$	$1.55 \pm 0.02^a$	$10.77 \pm 0.38^c$	$69.17 \pm 0.23^e$	$6.28 \pm 0.21^e$
0.2%	$23.13 \pm 0.86^d$	$2.70 \pm 0.01^b$	$11.33 \pm 0.15^d$	$63.03 \pm 2.82^d$	$5.32 \pm 0.19^d$
0.3%	$17.64 \pm 0.37^c$	$3.37 \pm 0.04^{cd}$	$9.75 \pm 0.24^b$	$38.14 \pm 1.76^c$	$3.53 \pm 0.09^c$
0.5%	$10.59 \pm 0.11^b$	$3.54 \pm 0.03^d$	$6.94 \pm 0.04^a$	$19.66 \pm 0.47^b$	$2.32 \pm 0.08^b$
0.7%	$9.19 \pm 0.01^a$	$3.33 \pm 0.00^{bc}$	$6.73 \pm 0.01^a$	$15.79 \pm 0.01^a$	$1.85 \pm 0.01^a$
Day 30					
0.05%	$30.53 \pm 0.16^f$	$1.46 \pm 0.01^a$	$10.70 \pm 0.61^c$	$80.70 \pm 0.41^f$	$7.08 \pm 0.41^f$
0.1%	$26.59 \pm 0.22^e$	$1.58 \pm 0.02^a$	$11.2 \pm 0.39^c$	$71.93 \pm 0.24^e$	$6.29 \pm 0.21^e$
0.2%	$23.88 \pm 0.95^d$	$2.74 \pm 0.01^b$	$11.70 \pm 0.21^d$	$65.17 \pm 2.76^d$	$5.33 \pm 0.17^d$
0.3%	$17.95 \pm 0.39^c$	$3.79 \pm 0.04^{cd}$	$9.95 \pm 0.25^b$	$38.80 \pm 1.8^c$	$3.53 \pm 0.09^c$
0.5%	$10.68 \pm 0.11^b$	$3.56 \pm 0.03^d$	$6.93 \pm 0.14^a$	$19.58 \pm 0.31^b$	$2.31 \pm 0.07^b$
0.7%	$9.15 \pm 0.09^a$	$3.31 \pm 0.03^{bc}$	$6.70 \pm 0.05^a$	$15.63 \pm 0.01^a$	$1.84 \pm 0.02^a$

The mean value followed by the same letter in the same column shows no significant difference with Duncan's test ( $p > 0.05$ )

Li *et al.* conducted a study using TEMPO–NFC from empty palm oil bunches to stabilize a dodecane-water emulsion with an oil : water phase ratio of 20 : 80 [20]. The study showed that the emulsion droplet diameter significantly decreased sharply when the TEMPO-NFC concentration increased by 0.1 – 1.0%. It was found that the critical loading concentration of TEMPO-NFC to stabilize the emulsion was around 0.8%. Furthermore, the droplet diameter increased slightly after

storage in two days compared to a fresh emulsion. The fresh emulsion's droplet size ( $d_{43}$ ) ranged from 10-18  $\mu\text{m}$ .

NFC-stabilized emulsions and particles generally have larger droplets than those stabilized by surfactants or other biomolecules such as proteins [1]. The particles are large and have a negligible thickness at the interface concerning the stabilized emulsion droplet size [17]. The particles have slower adsorption kinetics, a higher barrier potential against particle adsorption, and very high desorption energy. The slower adsorption kinetics shift the balance between re-coalescence and stabilization during droplet-droplet collisions and droplet particles during homogenization. Even though the particles are present, a high adsorption barrier reduces the particle attachment possibility to the interface during homogenization [29].

The span index (Table 1) shows the level of uniformity in the droplet size distribution pattern. In general, there is no acceptable limit for the span index, which depends on the purpose of forming the emulsion. Caetano *et al.* state that the droplet size distribution will be narrow if the span value is  $\leq 2.5$  [30]. A little distribution pattern shows that the particle size is homogeneous and vice versa; a broad distribution pattern exhibits that the particle size is heterogeneous. The emulsion droplet size distribution can be seen in Figure 2. The 0.05% and 0.1% pickering emulsion treatments had span values of around 7.08 and 6.24 with bimodal and multimodal droplet distribution patterns. Meanwhile, the monomodal distribution pattern was found in the 0.5% and 0.7% pickering emulsion treatment with span values of 2.32 and 1.85.

The  $D_{v10}$ ,  $D_{v50}$ , and  $D_{v90}$  values represent 10%, 50%, and 90% of the droplet size in the entire volume of the emulsion with diameters smaller or equal to the values. At concentrations of NFC  $\leq 0.2\%$ , the  $D_{v10}$  and  $D_{v90}$  values had a significant difference, with  $D_{v10}$  values ranging from  $1.43 \pm 0.01 \mu\text{m}$  to  $2.7 \pm 0.01 \mu\text{m}$  and  $D_{v90}$  values ranging from  $74.47 \pm 0.38 \mu\text{m}$  to  $63.03 \pm 2.82 \mu\text{m}$ . The significant difference indicates that the particle size distribution is broad or has a bimodal curve (Figure 2).

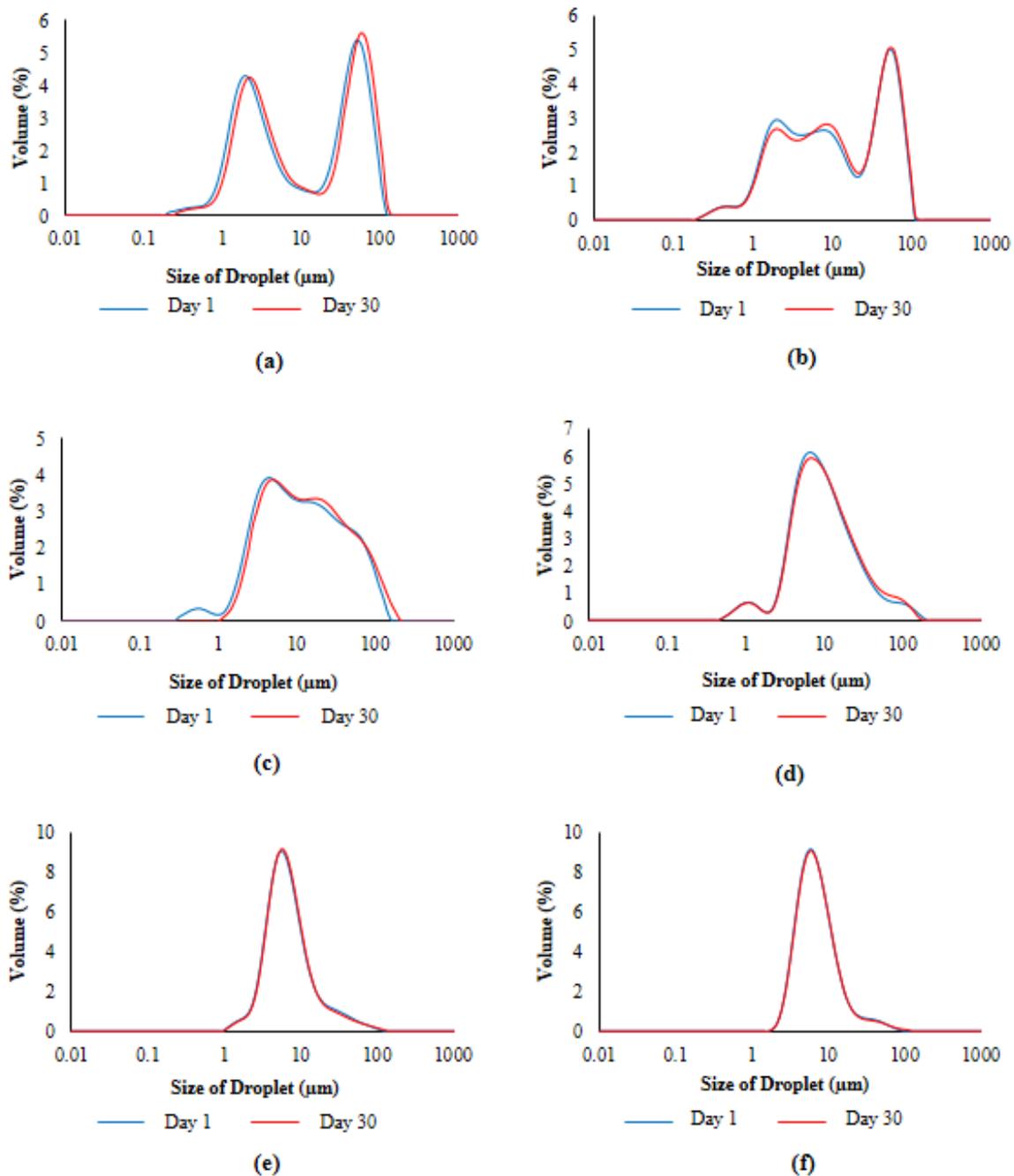


Figure 2. The effect of differences in NFC concentration on droplet size distribution on storage days 1 and 30, a) 0.05%, b) 0.1%, c) 0.2%, d) 0.3%, e) 0.5%, and f) 0.7 %.

### 3.3. Zeta Potential

Zeta potential is a parameter to determine the droplet surface charge magnitude in an emulsion. An improvement in the zeta potential value indicates the high droplet surface charge, so the repulsion between droplets increases. Formulations with high zeta potential values (more than  $\pm 30$  mV) provide good stability because they can prevent particle aggregation due to relatively high repulsive forces. Conversely, a low zeta value will result in greater attractive forces between particles and flocculation in the emulsion system [1].

The analysis of variance showed that differences in NFC concentrations significantly affected the zeta potential value ( $p < 0.05$ ), as presented in Table 2. The zeta potential values obtained ranged from -25.40 mV to -34.27 mV. Increasing the NFC concentration tends to increase the potential zeta value. The lowest zeta potential value was obtained at 0.05% pickering emulsion (-25.40 mV) and the highest at 0.7% pickering emulsion (-34.27 mV). The NFC layer covering the droplet surface contributes to the droplet's negative charge. NFC has a potential zeta value of  $-40.77 \pm 1.05$  mV. The NFC accumulation containing hydroxyl groups on the droplet surface impacts the negative charge on the droplet surface. This finding is similar to Li *et al.*, who obtained a potential zeta of -15 mV to -35 mV [31]. Meanwhile, Martins *et al.* obtained a higher potential zeta value, around -45 mV to -60 mV [14].

Table 2. The effect of differences in NFC concentrations on zeta potential

Pickering Emulsions	Zeta Potential (mV)
0.05%	$-25.40 \pm 1.23^a$
0.1%	$-30.83 \pm 0.6^b$
0.2%	$-32.07 \pm 0.71^{bc}$
0.3%	$-33.27 \pm 0.52^{cd}$
0.5%	$-33.60 \pm 0.44^d$
0.7%	$-34.27 \pm 0.401^d$

The mean value followed by the same letter in the same column shows no significant difference with Duncan's test ( $p > 0.05$ )

### 3.4. Rheological properties

The rheological measurements reflect the emulsion physicochemical properties under the external forces action [32]. The rheology of pickering emulsions exhibits flow and stability characteristics, as well as mechanisms of instability [4]. The pickering emulsion viscosity ( $\eta$ ) represents shear thinning behavior because its viscosity reduces when the shear rate improves, as shown in Figure 3.

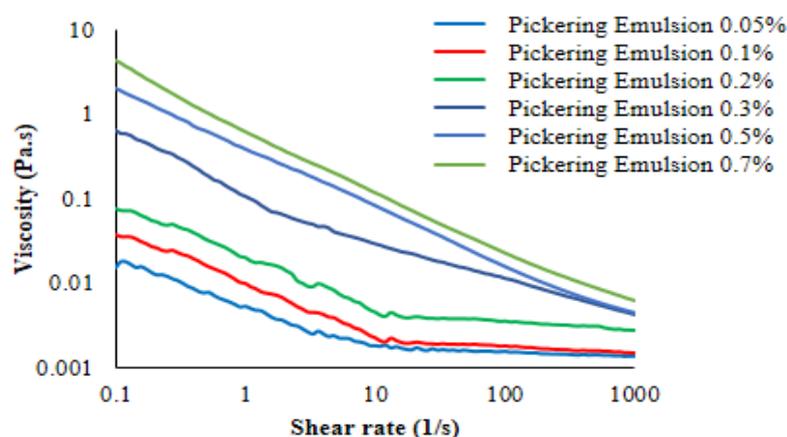


Figure 3. Relationship of shear rate (1/s) to viscosity (Pa.s)

Based on the analysis of variance, the effect of differences in NFC concentration on the viscosity value (at a shear rate in  $0.1 \text{ s}^{-1}$ ) showed a significant increase as the NFC concentration increased (Table 3). The particles are adsorbed at  $\text{NFC} \leq 0.3\%$  at the o-w interface. Increasing the concentration of NFC to 0.5% resulted in excess particles being dispersed in the dispersing phase to form internal bonds and interact strongly. This mechanism leads to an improvement in the emulsion viscosity, which limits the movement of the droplet to increase the emulsion stability. This increase in viscosity is also because of the high aspect ratio of NFC and the NFC flexibility, which forms an entangled network structure in the dispersing phase [33]. Previous research has shown that high emulsion viscosity can reduce the droplets floating speed and reduce collisions between droplets, thereby increasing the stability of the emulsion [34]. Figure 4 shows that shear stress improves with increasing shear rate, so emulsions stabilized by high NFC have a more entangled network structure that can withstand deformation [35].

Table 3. The effect of NFC concentration on the viscosity values  $\eta$ ,  $n$ ,  $K$ , and  $R^2$

Pickering Emulsion	$\eta$ (Shear Rate at $0.1 \text{ s}^{-1}$ )	Flow Behavior Index ( $n$ )	Viscosity Coefficient ( $K$ ) ( $\text{Pa}\cdot\text{s}^n$ )	$R^2$
0.05%	$0.015 \pm 0.00^a$	$0.73 \pm 0.03^a$	$0.006 \pm 0.00^a$	$0.97 \pm 0.01^{bc}$
0.1%	$0.038 \pm 0.01^a$	$0.63 \pm 0.02^b$	$0.010 \pm 0.00^a$	$0.95 \pm 0.01^a$
0.2%	$0.077 \pm 0.01^a$	$0.62 \pm 0.02^b$	$0.021 \pm 0.01^a$	$0.96 \pm 0.02^{ab}$
0.3%	$0.633 \pm 0.01^b$	$0.47 \pm 0.01^c$	$0.122 \pm 0.02^b$	$0.97 \pm 0.00^{ab}$
0.5%	$1.992 \pm 0.01^c$	$0.32 \pm 0.02^d$	$0.392 \pm 0.01^c$	$0.99 \pm 0.00^c$
0.7%	$4.31 \pm 0.11^d$	$0.28 \pm 0.02^d$	$0.646 \pm 0.01^d$	$0.98 \pm 0.01^{bc}$

The mean value followed by the same letter in the same column shows no significant difference with Duncan's test ( $p > 0.05$ )

Quantitatively, Newtonian and non-Newtonian flow patterns can be explained by the power-law model based on the relationship between shear stress and rate (Figure 4). The determination coefficient of the relationship between shear rate and stress is shown in Table 3. The results showed that the power-law model described the flow behavior of the pickering emulsion with an  $R^2$  of around 0.95 to 0.99, a value close to 1. The analysis of variance showed that the difference in NFC concentration significantly affects the flow behavior index ( $n$ ) and consistency index ( $K$ ). Increasing NFC concentration aligns with improving the  $K$  value as an emulsion viscosity parameter. The  $K$  value increased from  $0.006 \pm 0.00 \text{ Pa}\cdot\text{s}^n$  to  $0.646 \pm 0.01 \text{ Pa}\cdot\text{s}^n$ . This finding is similar to the study of Winuprasith and Suphantharika, that there was an increase in the value of  $K$  from  $0.02 \text{ Pa}\cdot\text{s}^n$  (MFC 0.05%) to  $0.43 \text{ Pa}\cdot\text{s}^n$  (MFC 0.5%) [29]. Meanwhile, Jutakradsada *et al.* reported that the  $K$  value increased from  $0.104 \text{ Pa}\cdot\text{s}^n$  (CNC-ammonium persulphate 0.1 wt%) to  $0.278 \text{ Pa}\cdot\text{s}^n$  (CNC-ammonium persulphate 0.5 wt%) [36]. Furthermore, the value of  $n$  tends to decrease as the NFC concentration increases, indicating that the shear-thinning behavior becomes

more pronounced at high NFC concentrations [29]. An emulsion stabilized by 0.7% NFC with a smaller  $n$  value indicates that the droplets may be more prone to slipping and rolling [35]. In addition, all emulsions in the flow behavior index ( $n$ ) showed pseudoplastic non-Newtonian behavior with a value of  $n < 1$  [35].

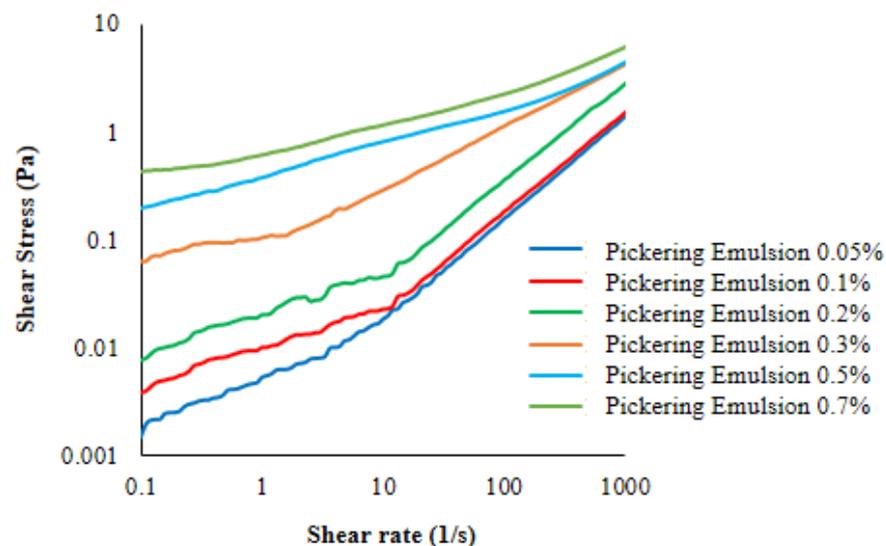


Figure 4. Relationship between shear rate (1/s) and shear stress (Pa)

### 3.5. Microscopic observation

Visualization of droplet micrograph observations at 200X magnification can be seen in Figure 5. Optical microscope observations show that the droplets are spherical with various sizes. This effect is under the droplet size distribution (Table 1), where a wide range is obtained between  $D_{10}$  and  $D_{90}$ . This effect was found at pickering emulsion 0.05% and 0.1%. Pickering emulsion treatment of 0.3% to 0.7% clearly shows droplet clusters, as shown in the figure marked by a red arrow. The formation of these droplet clusters tends to occur in NFC-stabilized emulsions, which is associated with the high aspect ratio of NFC particles [37, 14]. Previous researchers have widely reported this clustered structure using the terminology of networking organization [38] and droplet flock [39]. Based on the thermodynamic aspect, the formation of droplet clusters leads to depletion stabilization [38, 39]. Depletion stabilization occurs when stabilizer particles not adsorbed at the droplet interface form a 3D network structure that can inhibit droplet movement [40]. Lu *et al.* state that two essential routes exist to form droplet clusters. In the first route, the tiny droplets adhere around the large droplets' surface to form a planet-like structure [37]. Route two, the flexibility of NFC particles to link multiple droplets themselves and the simultaneous emulsification of droplets connected to different fiber sections can form grape cluster-like structures (marked by red arrows). The formation of grape cluster-like structures is visible in Figure 6.

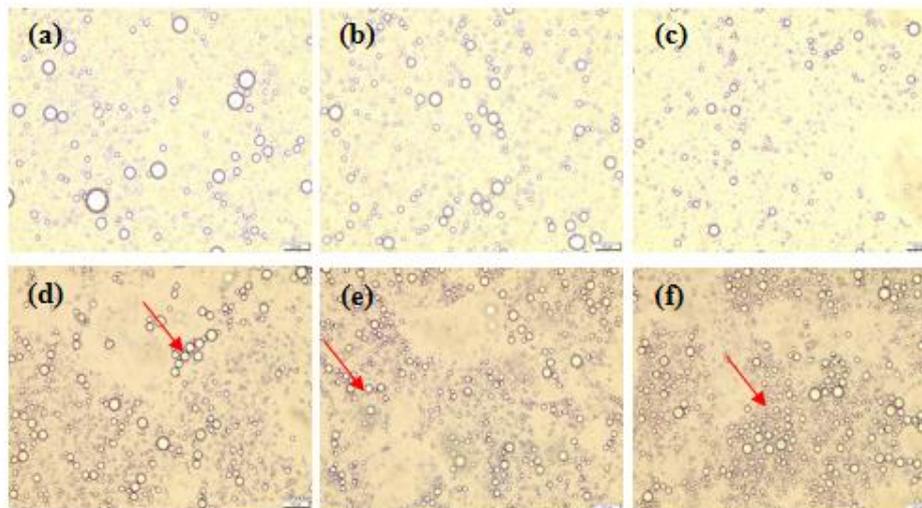


Figure 5. Pickering emulsion droplets micrograph at NFC concentrations of a) 0.05%, b) 0.1%, c) 0.2%, d) 0.3%, e) 0.5%, dan 0.7%



Figure 6. Formation of a grape cluster-like structure

### 3.6. Stability to coalescence and creaming

#### 3.6.1. Effect on coalescence

The mean comparison and droplet size distribution between emulsions stored for 1 and 30 days are shown in Table 1 and Figure 1. There was no dramatic change in  $d_{43}$  with storage time, except for  $d_{43}$  of the emulsion, which was stabilized by a low concentration of NFC (0.05 -0.10%). The emulsion showed no change in droplet size distribution upon 30 days of storage (Figure 1). The effects imply negligible droplet coalescence during the storage period. As is known, high resistance to coalescence is the primary function of solid particle-stabilized emulsions [27]. Medium wettability particles are effectively and irreversibly adsorbed at the o-w interface because the free adsorption energy is very high compared to the thermal energy. These adsorbed solid particles act as a steric (mechanical) barrier against coalescence. Therefore, the emulsion can be stabilized; the particles are very stable to coalescent even when the droplet size of the emulsion is quite large. These results agree with CNC-stabilized o-w emulsions [41] and chitin nanocrystals

[42], in which the average oil droplets particle size did not show any change after at least one month of storage, regardless of solid particle concentration. Meanwhile, Liu *et al.* obtained a stable pickering emulsion without any creaming process and phase separation after being stored at room temperature for 3 months [24].

### 3.6.2. Effect on creaming

The CI was measured by observing the serum level at a particular time without centrifugation. This effect is because this study evaluated the pickering emulsion stability against the CI based on the gravitational force on the droplets during 30 days of storage. The NFC concentrations' effect on the CI is presented in Table 4. The results of the variance showed that differences in NFC concentrations had a significant effect on the CI.

Table 4. Effect of NFC on CI%

Pickering Emulsions	Creaming Index (CI) %			
	Day 1	Day 10	Day 20	Day 30
0.05%	00.00	12.22 ± 0.88 <sup>a</sup>	17.81 ± 1.17 <sup>a</sup>	24.14 ± 1.07 <sup>a</sup>
0.1%	00.00	6.01 ± 1.00 <sup>b</sup>	13.08 ± 1.76 <sup>b</sup>	20.97 ± 0.49 <sup>b</sup>
0.2%	00.00	2.27 ± 0.53 <sup>c</sup>	9.10 ± 1.19 <sup>c</sup>	16.59 ± 0.96 <sup>c</sup>
0.3%	00.00	00.00	3.57 ± 0.21 <sup>d</sup>	7.27 ± 0.59 <sup>d</sup>
0.5%	00.00	00.00	00.00	00.00
0.7%	00.00	00.00	00.00	00.00

The mean value followed by the same letter in the same column shows no significant difference with Duncan's test ( $p > 0.05$ )

Emulsions stabilized by NFC at low concentrations ( $\leq 0.10\%$ ) tend to form a creaming process. In contrast, stabilizing the emulsion with  $\text{NFC} \geq 0.50\%$  did not form a cream for 30 days. The CI's measurement has results that align with the droplet diameter measurements and the pickering emulsion's viscosity, where an increase in the NFC concentration generates a smaller droplet size and increases the viscosity. This effect causes the emulsion to have high stability based on the CI, which is zero at a concentration of  $\text{NFC} \geq 0.50\%$ . Based on Stoke's law, the emulsion creaming stability can be increased by reducing the droplet size, improving the dispersing phase viscosity, or minimizing the difference in density between the droplet and the dispersing phase [26].

At concentrations of NFC higher than 0.50% and 0.70%, the emulsion had tiny droplets, resulting in very high stability against creaming during 30 days of storage. It can be attributed to depletion-flocculation networks, 3D droplet networks of NFC particles formed in excess non-adsorbed NFC particles. In summary, emulsions containing a high concentration of NFC (0.50%) show good stability against creaming because of their gel-like structure.

Creaming or flocculation generally does not indicate instability unless associated with droplet coalescence because soft stirring can easily redisperse emulsion droplets [3, 33]. However,

creaming often precedes flocculation, coalescence, and droplet size distribution evolution over time [43].

#### 4. Conclusions

The concentration of NFC isolated from OPEFB significantly affects the characteristics and stability of the pickering emulsion. NFC concentration  $<0.5$  w/v% produces emulsion with large droplets, whereas at NFC concentration  $\geq 0.5$  w/v%, it forms smaller emulsion droplets. The concentration of NFC is related to the total of particles adsorbed on the droplet's surface in the dispersed phase. The higher the particle concentration, the more particles are adsorbed at the o-w interface. Furthermore, increasing the particle concentration also formed 3D droplet networks from excess or non-adsorbed NFC particles at the o-w interface. The 3D network formation increases the pickering emulsion viscosity. Furthermore, the high viscosity will inhibit the movement of droplets and form a more stable emulsion. However, it is necessary to carry out further tests such as oxidation stability, storage time, the influence of pH and salt, gravity, and heating.

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