



# Food and Applied Bioscience Journal



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## Properties and stability of tween 20-stabilized emulsions containing nanocellulose

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### Abstract

This study presented the influence of the addition of nanocellulose, either nanocrystalline cellulose (NCC) or nanofibrillated cellulose (NFC), on the stability and properties of Tween 20-stabilized 10% oil-in-water (O/W) emulsions including the particle size, viscosity, particle charge, color and creaming index. In this research, Tween 20 was used as an emulsifier whereas either NCC or NFC was used as a stabilizer at 0.05% (w/w), 0.10% (w/w), and 0.20% (w/w). The result of the particle size showed that emulsion containing NFC at all concentrations had a bigger droplet size than the others due to the bridging flocculation induced by the long entanglement structure of the NFC itself. Additional NFC at a concentration of 0.20% (w/w) significantly increased ( $P < 0.05$ ) the viscosity of the continuous phase of the emulsion. Either emulsion containing NCC or NFC exhibited a higher negative charge than the control emulsion as a result of providing higher emulsion stability due to higher electrostatic repulsion between the oil droplets. The differences between the emulsions stabilized by the nanocellulose on the color with the control emulsion were not noticeable by visual observation, thereby they were suitable for their intended use. As creaming was often used as a precursor for other physical instability, especially flocculation and coalescence, thereby 0.20% (w/w) of NFC was the most preferable against the creaming index than others. This could be attributed to the larger droplet size, higher viscosity, and higher negativity charge. This research would provide useful information for further applications in the field of a colloidal delivery system for bioactive compounds.

**Keywords:** Emulsion, nanocrystalline cellulose, nanofibrillated cellulose, stability

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## 1. Introduction

Emulsion is a dispersed system of two immiscible liquids, which one liquid is dispersed as small spherical droplets on the other (Dickinson, 2009; McClements, 2005). Emulsion also consists of the continuous and aqueous phases. The continuous phase is the external phase where the droplet is dispersed, and the aqueous phase is the internal phase or the dispersed phase (Chrisman et al., 2012). Moreover, emulsion is classified according to the relative spatial distribution of the oil and aqueous phases comprising the oil-in-water (O/W) and water-in-oil (W/O) emulsions. The emulsions can easily become unstable, especially from physical instability during the storage time; however, the duration that the emulsions remain stable would depend on the nature of the food products (Dickinson, 2009). The normal physical instability includes creaming, sedimentation, flocculation, and coalescence (Liang et al., 2014). To produce more stable emulsions, an emulsifier and a stabilizer are needed. An emulsifier is a substance that facilitates the formation of stable emulsions by lowering the interfacial tension by forming a protective film around the droplets. Moreover, the addition of an emulsifier can promote a smaller droplet size in the emulsion (Xu et al., 2017). Generally, each kind of emulsifier has the particular function such as in Tween 20 can be used to promote a smaller droplet size due to the higher value of the hydrophilic lipophilic balance (HLB) (Griffin., 1949). On the other hand, a stabilizer is a texture modifier and thickening agent with the primary function to increase the viscosity of the continuous phase of the emulsions in order to reduce the rate of the particle sediment or cream; such as, gums, gelatin, cellulose, and modified starch (Mitchell et al., 1986). Gelatin is commonly utilized as a gelling agent and also used as an additive in food, cosmetics, drugs, and foam stabilizer (Damin et al., 2009). The process of the extraction of gelatin from collagen has been developed by incomplete hydrolysis of collagen obtained from the skin, and connective tissues of animals. Another common stabilizer in emulsion is sodium caseinate, which acts with higher viscosity and a stronger network (Ares et al., 2007). However, these common stabilizers are animal-based and protein-based where it is easier for the denaturation or coagulation by several conditions; such as, by a high temperature or high acidic condition.

Nowadays, consideration is given to using natural plant-based than synthetic or animal-based food ingredients. Nanocellulose has attracted more attention because of some unique characteristics; such as, low density, sustainability, biodegradability, and low cost (Kalashnikova et al., 2011; Turbak et al., 1983). The nanocellulose comprises nanoscale (1–100 nm) materials with highly uniform material that can be extracted from various types of agricultural by-products using chemical, enzymatic, and mechanical methods (Isogai, 2013). There are three types of nanocellulose. First, nanocrystalline cellulose (NCC) is commonly produced by using acid hydrolysis where it hydrolyzes the amorphous regions of cellulose, thereby obtaining the crystalline regions only (Lu and Hsieh, 2012). Second, nanofibrillated cellulose (NFC) is present as interconnected fibers with diameters in the nanometer range, but lengths of several micrometers or more (Winuprasith et al., 2018). The NFC contains both amorphous and crystalline cellulose domains within the single fibers (Jiang and Hsieh, 2013). Third, bacterial cellulose (BC) is typically produced from bacteria; such as, *Acetobacter xylinum* (Winuprasith et al.,



2017; Lin et al., 2013). The nanocellulose can also be called cellulose gel because it exhibits a gel-like behavior (Winuprasith and Suphantharika, 2013). In addition, nanocellulose has been found as applications as a stabilizer and rheology modifiers in food, paint, cosmetics, and pharmaceutical products (Choublab and Winuprasith, 2019; Issara and Rawdkuen, 2017; Turbak et al., 1983). Another ability of nanocellulose is both NCC and NFC can form and stabilize O/W emulsions through a "Pickering mechanism" by forming a protective steric barrier around the oil droplets (Winuprasith et al., 2015). This was also supported by Kalashnikova et al. (2013) that NCC stabilized emulsion could increase the interfacial tension of O/W, which led to a clear decrease of the mean diameter of the emulsion; as a result, the emulsion displayed excellent mechanical resistance against coalescence. Moreover, due to the strong entangled and disordered network, it may increase the viscosity of the continuous phase of the emulsion, as a result of enhancing the stability of the emulsion (Paakko et al., 2007).

Previous research (Bai et al., 2019; Winuprasith et al., 2015) investigated the ability of NFC as Pickering emulsions only, and another study explained about NCC as an emulsifier in emulsion. However, both studies showed the ability of nanocellulose as an emulsifier, and there was a lack of knowledge describing and comparing the efficiency of NCC and NFC as a stabilizer in the O/W emulsion system. The effect of the nanocellulose type (NCC and NFC) and concentration (0.05% (w/w), 0.10% (w/w), and 0.20% (w/w)) on the emulsion properties and stability were examined. These studies had important implications for the utilization of a natural plant-based stabilizer in the development of label-friendly emulsions.

## 2. Materials and Methods

### 2.1 Materials

Suspensions of 12.2% (w/w) nanocrystalline cellulose (NCC) and 3% (w/w) nanofibrillated cellulose (NFC) were purchased from Cellulose Lab Company (Canada). Tween 20 was purchased from Sigma-Aldrich Company. Soybean oil used for preparing the emulsion was purchased from a local supermarket in Thailand and used without further purification. All chemicals used in this experiment were an analytical grade. Double distilled water was used for the preparation of all solutions.

### 2.2 Emulsion preparation

Oil-in-water (O/W) emulsion was prepared by using the well-established two-step procedure (Zhang et al., 2016). The oil phase was prepared by mixing 1% (w/w) of tween 20 and soybean oil. The continuous phase consisted of 0.01% (w/w) of sodium azide as an anti-microbial agent, and buffer solution (10 mM of sodium phosphate, pH 7).

Coarse emulsion was prepared by mixing 10% (w/w) oil phase and 90% (w/w) aqueous phase together using a high-shear mixer (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) for two min at room temperature (25°C). Then, the coarse emulsion was ultrasonicated using an ultrasonicator (Model Intelligent Ultrasonic Processor, Nanjing Safer Biotech Co, Nanjing, China) for five minutes by using probe No. 6 with a power rate of 50% in order to obtain the fine emulsion.

Either NCC or NFC was added to obtain emulsion at concentration of 0.05% (w/w), 0.10% (w/w), and 0.20% (w/w). Then, the final emulsion with nanocellulose was obtained after mild stirring at room temperature (25°C) for one h to completely dissolve the nanocellulose in the emulsions.

### 2.3 Determination of the particle size and size distribution

The particle size and size distribution of the freshly prepared emulsions were measured by using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, United Kingdom) (Jain *et al.*, 2016). The samples were diluted in 10 mM of phosphate buffer solution (pH 7) to avoid multiple scattering effects, and then the rate of the stirrer was controlled at 1,200 rpm until achieving an obscuration rate of 10–15%, respectively. The refractive indices of the oil and water phases used in the calculations were 1.46 and 1.33, respectively. The particle diameter was reported as the surface-weighted mean diameter ( $d_{32}$ ) and volume-weighted mean diameter ( $d_{43}$ ), which were calculated from the full particle size distribution.

### 2.4 Determination of the Viscosity

The viscosity of the freshly prepared emulsions was measured at 25°C using a controlled-strain rheometer (Physica MCR 301, Anton Paar GmbH, Graz, Austria) (Winuprasith *et al.*, 2013). Equipped with a cone and plate sensor (1° cone angle, 50 mm diameter, and 0.05 mm gap), the viscosity information was obtained from steady flow tests. The measuring sensor was programmed to a linear increase shear rate from 0.1 s<sup>-1</sup> until 300 s<sup>-1</sup> in three minutes followed immediately by a reduction from 300 s<sup>-1</sup> to 0.1 s<sup>-1</sup> in the next three min. The temperature of the measurement was controlled at 25°C.

### 2.5 Determination of the ζ-potential

The particle charge of the freshly prepared emulsions was measured using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, United Kingdom) (Zhang *et al.*, 2015). The samples were diluted using deionized water to avoid multiple scattering effects. The particle charge was reported as the average and standard deviation of the measurements made on three freshly prepared samples, with two readings taken per sample.

### 2.6 Determination of the color

The freshly prepared emulsions were measured for color in the  $L^*$ ,  $a^*$ , and  $b^*$  systems using a Hunter Lab Digital Colorimeter (CR-20 Color Reader, Konica Minolta Sensing Americas, Inc., Williams Ramsey, United States) (Jain *et al.*, 2019). First, the colorimeter was calibrated using a white standard porcelain plate ( $L^* = 97.10$ ,  $a^* = -0.07$ ,  $b^* = +1.97$ ). Next a fixed amount of the emulsion was poured into a measurement cup, which was then surrounded with a black cover. In this color system,  $L^*$  represented the lightness, and  $a^*$  and  $b^*$  were the color coordinates: where +a was the red, -a was the green, +b was the yellow, and -b was the blue directions (Burapalit *et al.*, 2020). The total color difference ( $\Delta E$ ) was measured the following equation (Ping *et al.*, 2019):

$$\text{The total color difference } (\Delta E) = \sqrt{(L^* - L_i^*)^2 + (a^* - a_i^*)^2 + (b^* - b_i^*)^2}$$

Where,  $L^*$ ,  $a^*$ ,  $b^*$  are the color coordinates measured at time  $t$ , and  $L_i^*$ ,  $a_i^*$  and  $b_i^*$  are the color coordinates measured initially.

## 2.7 Determination of the creaming index

Thirteen grams of freshly prepared emulsions were transferred into transparent glass test tubes 20 mm in diameter and 70 mm in height) then sealed with a plastic cap (Winuprasith *et al.*, 2013). The sample tubes were kept at room temperature (25°C) and the separation of the creaming boundary was observed every week for 60 days and calculated as a creaming index. The creaming index (CI) was measured by the following equation:

$$\text{CI (\%)} = (H_s/H_T) \times 100$$

Where,  $H_T$  is the total height of the emulsion and  $H_s$  is the serum phase height.

## 2.8 Statistical analysis

All measurements were performed in triplicate. The data were reported as the mean  $\pm$  standard deviation. A one-way analysis of variance (ANOVA) with Duncan's multiple range test was used to indicate the significant differences ( $P < 0.05$ ) between the effect of the nanocellulose type and concentration on each parameter of the stability of the emulsion. The statistical analysis was performed using the commercial software of the SPSS version 19.0 Windows program.

# 3. Results and Discussion

## 3.1 Effect of NCC and NFC on the particle size of the emulsions

The influence of the type of nanocellulose and concentration on the surface weighted mean diameter ( $d_{32}$ ) and volume weighted mean diameter ( $d_{43}$ ) of the tween 20-stabilized 10% (w/w) O/W emulsions are shown in Table 1. There were no significant differences ( $P \geq 0.05$ ) between the  $d_{32}$  of the control emulsion and emulsions containing NCC, which exhibited a smaller particle size than those containing NFC. The  $d_{32}$  of the emulsions containing the NFC significantly increased ( $P \geq 0.05$ ) from 1.15  $\mu\text{m}$  to 1.36  $\mu\text{m}$  by increasing the concentration of the NFC from 0.05% (w/w) to 0.20% (w/w). In addition, the  $d_{43}$  of the emulsions containing the NFC at all concentrations were significantly higher ( $P < 0.05$ ) compared with the others. The different results of the particle size on the emulsion containing the NCC and NFC may have occurred because of flocculation in the emulsions containing the NFC (Mitbunrung *et al.*, 2019). This phenomenon was clearly supported by the  $d_{43}$  values.

McClements (2015) stated that generally the  $d_{43}$  would be more sensitive to the presence of large particles within a polydispersed system, as this would be useful for detecting the small amount of flocculation or coalescence in the emulsion. The effect of NCC and NFC on the particle size would be different possibly because of their structure. The NCC has

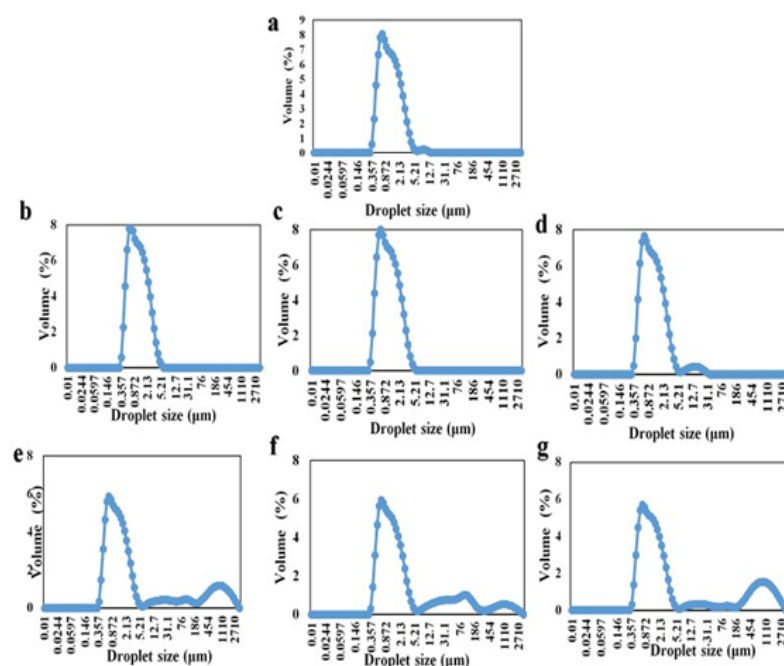
a short and rod shape structure (Kalashnikova et al., 2013), which is easily dispersed in the emulsions; therefore, it may help to prevent flocculation (Aulin et al., 2009). In contrast, the NFC has a long entanglement and is presented as interconnected long fibers (Winuprasith, et al., 2018) leading to the formation of porous and multilayered organizations. This would act as a bridge between the oil droplets, or so-called bridging flocculation phenomenon, which would provide good agreement to prevent the droplet from coalesce. This statement was clearly supported by the results of the particle size distribution (Fig 1).

**Table 1** Surface-weighted mean particle diameter ( $d_{32}$ ) and volume-weighted mean particle diameter ( $d_{43}$ ) of the tween 20-stabilized 10% O/W emulsions containing either NCC or NFC at various concentrations at 0.05, 0.10, 0.20% (w/w).

Emulsion sample	Concentration of nanocellulose (%)	$d_{43}$ ( $\mu\text{m}$ )	$d_{32}$ ( $\mu\text{m}$ )
Control	0	$2.80 \pm 0.14\text{c}$	$1.10 \pm 0.01\text{d}$
NCC	0.05	$2.60 \pm 0.21\text{c}$	$1.13 \pm 0.01\text{cd}$
	0.10	$2.65 \pm 0.29\text{c}$	$1.13 \pm 0.01\text{cd}$
	0.20	$2.54 \pm 0.23\text{c}$	$1.11 \pm 0.01\text{c}$
NFC	0.05	$5.68 \pm 0.35\text{c}$	$1.15 \pm 0.01\text{c}$
	0.10	$11.87 \pm 0.76\text{b}$	$1.31 \pm 0.02\text{ab}$
	0.20	$17.43 \pm 4.40\text{a}$	$1.36 \pm 0.04\text{a}$

**Note:** The assays were performed in triplicate. The mean  $\pm$  standard deviation values in the same column were followed by different lowercase letters (a–d) show the significant differences ( $P < 0.05$ ).





**Fig 1** The particle size distributions of the Tween 20-stabilized 10% (w/w) O/W emulsions without nanocellulose (a) and with nanocellulose, including 0.05% NCC (b), 0.05% NFC (c), 0.10% NCC (d), 0.10% NFC (e), 0.02% NCC (f), and 0.02% NFC (g).

For the full particle size distribution, the control emulsion (Fig. 1a) and emulsion with the addition of NCC at concentration 0.05% (w/w) and 0.10% (w/w) exhibited monomodal distribution with only one of single peak (Fig. 1 b-c) whereas the addition of NCC at a concentration 0.20% (w/w) promoted another peak. On the other hand, emulsions containing NFC at all concentrations exhibited multimodal distribution containing more than two peaks (Fig. 1 e-g), where, increasing concentration of NFC from 0.05 to 0.20% (w/w) promoted the wider particle size distribution. This occurred because of bridging flocculation by NFC (Chung et al., 2012) which could be confirmed by the result of the viscosity at a shear rate of  $100 \text{ s}^{-1}$  measured by a rheometer (Table 2). 3.2 Effects of the NCC and NFC on the viscosity of the emulsion

Table 2 shows that there were no significant differences ( $P \geq 0.05$ ) of the viscosity in the control emulsion and NCC at all concentrations. On the other hand, the viscosity of the emulsion containing NFC was progressively higher than those of the control and emulsions containing NCC, especially at 0.20% of NFC where it increased steeply from 0.0047 Pa.s to 0.8600 Pa.s. This result suggested that the synergetic effect on the concentration of the NFC and viscosity were strongly associated with each other due to the existing three dimensional network structures of the continuous phase, thereby preventing the droplets from aggregation and coalescence (Winuprasith and Suphantharika, 2015). Further insight was supported by McClements (2015) that was based on Stokes' law, which had higher viscosity of the continuous phase that could be used against creaming (Section 3.5).

**Table 2** Viscosity at a shear rate of  $100 \text{ s}^{-1}$  of the tween 20-stabilized 10% (w/w) O/W emulsions containing either NCC or NFC at 0.05, 0.10, and 0.20% (w/w) at  $25^{\circ}\text{C}$ 

Emulsion Sample	Concentration of Nanocellulose (%)	Viscosity (Pa.s)
Control	0	$0012.0 \pm 0.001\text{a}$
NCC	0.05	$0012.0 \pm 0.001\text{a}$
	0.10	$0.0013 \pm 0.001\text{a}$
	0.20	$0014.0 \pm 0.001\text{a}$
NFC	0.05	$0047.0 \pm 0.001\text{a}$
	0.10	$0062.0 \pm 0.001\text{a}$
	0.20	$8600.0 \pm 0.650\text{b}$

**Note:** The assays were performed in triplicate. The mean  $\pm$  standard deviation values in the same column were followed by different lowercase letters (a–b) show the significant differences ( $P < 0.05$ ).

### 3.3 Effect of NCC and NFC on the $\zeta$ -potential of the emulsion

Table 3 shows the magnitude of the  $\zeta$ -potential in the control emulsion ( $-19.80 \text{ mV}$ ) that was significantly lower ( $P < 0.05$ ) than those of the emulsions containing either NCC or NFC. This inferred that the addition of NCC and NFC from 0.05% to 0.20% (w/w),  $\zeta$ -potential of emulsion were increased up to  $-29.54 \text{ mV}$ . The low negativity charge of the control emulsion ( $-19.80 \text{ mV}$ ) could occur because this emulsion only consisted of Tween 20 where the Tween 20, which was a non-ionic surfactant (Nylander, 2004). Tween 20 would not be expected to give any charge due to the fact that it was classified as a non-ionic surfactant. Nevertheless, it has been shown that the control emulsion had an appreciable negative charge, which was attributed to the preferential adsorption of the hydroxyl ion ( $\text{OH}^-$ ) from the aqueous phase or the presence of anionic impurities during the preparation of emulsion (McClements, 2005). The  $\zeta$ -potential of the emulsions containing either NCC or NFC at 0.02% (w/w) were  $-29.26 \text{ mV}$  and  $-28.54 \text{ mV}$ , respectively. The NCC and NFC are anionic polysaccharides that promote electrostatic repulsive forces between the droplets (Winuprasith and Suphantharika, 2013). This was also supported by Mitbumrung et al., (2019) that the addition of 0.30–0.70% (w/w) NFC helped to increase the  $\zeta$ -potential ( $>25 \text{ mV}$ ) of the emulsions. In addition, the presence of sulfuric acid during the extraction of NCC exhibited the surface OH-groups of NCC partially react with the acid creating charged sulfate ester groups on its surface as the result of promoting the high magnitude of the  $\zeta$ -potential. The absolute value of the  $\zeta$ -potential higher than 25–30 mV could provide strong electrostatic repulsion between the oil droplets to overcome the attractiveness of Van der Waals interaction, thereby increasing the stability of the emulsion (Hunter, 1986).



**Table 3**  $\zeta$ -potential of the tween 20-stabilized 10% (w/w) O/W emulsions containing either NCC or NFC at 0.05, 0.10, and 0.20% (w/w).

Emulsion sample	Concentration of nanocellulose (%)	$\zeta$ -potential (mV)
Control	0	-19.80 $\pm$ 3.40b
NCC	0.05	-26.08 $\pm$ 0.44a
	0.10	-27.84 $\pm$ 0.51a
	0.20	-29.26 $\pm$ 0.47a
NFC	0.05	-28.77 $\pm$ 0.39a
	0.10	-29.24 $\pm$ 0.39a
	0.20	-29.54 $\pm$ 0.34a

**Note:** The assays were performed in triplicate. The mean  $\pm$  standard deviation values in the same column were followed by different lowercase letters (a–b) show the significant differences ( $P < 0.05$ ).

### 3.4 Effects of NCC and NFC on the color of the emulsion

Due to the relatively high color intensity of the NFC, it was worth examining the color of the emulsions to check if the product was suitable for daily use. The influence of the type of nanocellulose and concentration on the color of 10% (w/w) O/W of the emulsions is shown in Table 4. The color parameters, including the  $L^*$ ,  $a^*$ , and  $b^*$  values were examined. It was found that the value of  $L^*$  was significantly ( $P < 0.05$ ) different between the control emulsion and emulsion containing NCC and NFC. Increasing the concentration of the NCC and NFC decreased the  $L^*$  of the emulsions because of the yellow–brown color of the NFC and NCC that induced the emulsion to become darker. In addition, decreasing the  $L^*$  was related to the difference in the particle size of the control emulsion and the emulsions containing nanocellulose, especially NFC at a concentration of more than 0.05% (w/w).

The emulsions contained different ranges of the size of the oil droplets, so the light waves were scattered differently by the droplets in each size class where the larger droplet size could absorb more light, thereby decreasing the  $L^*$  value (Bohren and Huffman, 1983). Although, the color parameters measured by a colorimeter exhibited a significant difference, these differences were not noticeable by visual observation. This was further supported by the total color difference ( $\Delta E$ ) value lower than 1.00 indicating that human eyes could not detect the difference in color between the control and the emulsion containing nanocellulose, so it was assumed that the emulsions system was appropriate for the intended use (Hunter, 1986).

**Table 4** Color parameters of the tween 20-stabilized 10% (w/w) O/W emulsions containing either NCC or NFC at 0.05, 0.10 and 0.20% (w/w).

Emulsion sample	Concentration of nanocellulose (%)	$L^*$	$a^*$	$b^*$	$\Delta E$
Control	0	56.84 ± 0.04c	-0.17 ± 0.01a	0.26 ± 0.01ab	-
NCC	0.05	56.93 ± 0.01a	-0.15 ± 0.01b	0.28 ± 0.01a	0.43
	0.10	56.95 ± 0.06a	-0.16 ± 0.01ab	0.22 ± 0.01ab	0.54
	0.20	56.87 ± 0.04b	-0.17 ± 0.01a	0.24 ± 0.04cd	0.65
NFC	0.05	56.87 ± 0.02b	-0.15 ± 0.01b	0.25 ± 0.01bc	0.32
	0.10	55.80 ± 0.03d	-0.16 ± 0.01ab	0.25 ± 0.01d	0.87
	0.20	55.88 ± 0.01e	-0.13 ± 0.01c	0.26 ± 0.01ab	0.76

**Note:** The assays were performed in triplicate. The mean ± standard deviation values in the same column were followed by different lowercase letters (a–e) show the significant differences ( $P < 0.05$ ).

### 3.5 Effect of NCC and NFC on the creaming index of the emulsion

The influence of the type and concentration of nanocellulose on the creaming index of 10% (w/w) O/W emulsions is shown in Table 5. After storage for 60 days, the phase separation occurred in all emulsions. The control emulsion had the highest creaming index (7.92%). It could be clearly seen that the lower creaming indices were found in the emulsions containing nanocellulose. The creaming indices decreased when the nanocellulose concentration was increased. In addition, the lowest creaming index was found in the emulsions containing 0.20% of NFC (0.92%); moreover, even the results from particle size ( $d_{43}$ ) indicated that there was some flocculation occurring in the emulsions containing NFC, especially at higher concentrations. The addition of the NFC dramatically caused an increase in the viscosity of the emulsions that effectively helped to slow down the oil droplet movement upward, thereby decreasing the phase separation. As mentioned earlier, the author would have expected a good creaming stability of the emulsions because of the relatively high electrostatic repulsion, i.e., high absolute values of the  $\zeta$ -potential (Sadeghifar et al., 2011; Winuprasith, 2015). Therefore, increasing the concentration of the NFC up to 0.20%, it could be assumed that this would have an extremely long shelf life due to the slower rate of the creaming index because creaming was often used as a precursor for other physical instability, especially flocculation and coalescence (Rayner et al., 2014).



**Table 5** Creaming index (%) of the tween 20-stabilized 10% (w/w) O/W emulsions containing either NCC or NFC at 0.05, 0.10, and 0.20% (w/w) after storage at room temperature (25°C) for 60 days.

Emulsion Sample	Concentration of Nanocellulose (%)	Creaming Index (%)
Control	0	92.7± 0.72a
NCC	0.05	6.17 ± 0.14b
	0.10	83.5 ± 0.72b
	0.20	83.5± 0.72b
	0.05	25.1 ± 0.01c
NFC	0.10	00.1 ± 0.66d
	0.20	92.0± 0.38e

**Note:** The assays were performed in triplicate. The mean ± standard deviation values in the same column were followed by different lowercase letters (a–e) show the significant differences ( $P < 0.05$ ).

#### 4. Conclusion

In this study, the effect of the types of nanocellulose, comprising nanocrystalline cellulose (NCC) and nanofibrillated cellulose (NFC), at concentrations of 0.05–0.20% (w/w) on the emulsion properties and stability were studied. Control emulsion and emulsion containing NCC exhibited a smaller particle size. On the other hand, the addition of NFC caused an increase in the particle size of the emulsion by increasing the concentration from 0.05% to 0.20% (w/w) due to the bridging flocculation phenomenon. The highest concentration of NFC increased the viscosity of the continuous phase by approximately 700 times. Both the NCC and NFC had a higher negative charge on their surfaces than the control emulsion, which increased the electrostatic repulsion between the oil droplets resulting in more stable emulsions. Increasing the concentration of NCC from 0.05% (w/w) to 0.20% (w/w) was more preferable as a colloidal delivery system due to the smaller particle size, thereby increasing a higher surface area. This was due to creaming that was often used as a precursor for other physical instability, especially flocculation and coalescence, thereby 0.20% (w/w) of NFC was the most preferable as a stabilizer because it could slow down the creaming index better than the NCC and control emulsions. This was caused by an increase in the particle size (bridging flocculation phenomenon), continuous higher viscosity, and higher negativity charge.

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