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Physical properties of beeswax-oleic acid mixture nanoemulsions as affected by lipid ratio and concentration of emulsifier

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Abstract

Lipid nanoemulsion is an effective system for improving or extending the functional performance of a composite coating or film since the small particle size causes the lipids to be evenly dispersed into the matrix. This study aimed to examine the effect of the ratio of BW: OA and the concentration of Tween 80 on the physical characteristics of the resulting lipid nanoemulsion. Lipid mixture nanoemulsion was prepared from beeswax (BW) and oleic acid (OA) in various ratios, of 100:0, 75:25, 50:50, and 25:75. Tween 80 as a surfactant was added at concentrations of 2.5%, 5.0%, 7.5%, and 10%. The characteristics of the nanoemulsion were determined by measuring the viscosity, particle size, polydispersity index (PDI), zeta-potential, and stability of the nanoemulsion. Lipid ratio BW:OA and surfactant concentration greatly affected the viscosity, particle size, PDI, zeta-potential, and stability of nanoemulsions. At a higher BW amount than oleic acid, increasing the concentration of Tween 80 did not affect the nanoemulsion viscosity, but decreased the particle size, PDI value, and zeta-potential value. On the other hand, in nanoemulsions with an OA ratio greater than BW, an increase in Tween 80 increased the viscosity, particle size, and PDI value. Nanoemulsions with small particle sizes did not undergo separation when tested by the Freeze-Thaw method. These results showed that the particle size and PDI values determined the stability of the nanoemulsion. From this study, it was found that formulation largely determined the characteristics of nanoemulsions.

1. Introduction

The use of edible coatings or films in the post-harvest handling of fruits has very potential. Fruit coating can control water vapor transfer, respiration rate, oxidation processes, flavor components, and extend shelf life (Hassan *et al.,* 2018; Joshi and Rao, 2018). The main components of edible coatings are polysaccharides, proteins, and lipids (Sothornvit and Krochta, 2001; Vargas *et al*., 2008). Many studies suggest that hydrocolloid-based edible coatings are able to maintain volatile components, and control gas exchange in minimally processed or fresh fruit (Marpaung *et al.,* 2015; Alali *et al.,* 2018; Nor Amalini *et al.,* 2018; Yousuf *et al.,* 2018; Thakur *et al*., 2019). However, since they are hydrophilic, natural biopolymers coating are poor barrier materials that prevent water loss of product (Arnon *et al.,* 2014; Hassan *et al.,* 2018). On the other hand, lipids are better in moisture barrier (Pérez-Gago and Krochta, 2001). The functional properties of the edible coating can be enhanced by adding hydrophobic lipids into protein or polysaccharide-based coating (Zhang *et al.,* 2018).

Various studies have been carried out to reduce water vapor permeability (WVP) of coatings by incorporation of lipids (Kowalczyk and Baraniak, 2014; Khanzadi *et al.,* 2015; Klangmuang and Sothornvit, 2016). According to Zhang *et al.* (2018), beeswax is more effective than carnauba wax in reducing the WVP of edible films because it has high hydrophobicity. In addition, beeswax also contains antimicrobials and antioxidants (Fratini *et al.,* 2016). The addition of beeswax significantly reduces the WVP film based on

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hydroxypropyl methylcellulose (HPMC) (Klangmuang and Sothornvit, 2016). However, although the addition of beeswax lowers the WVP and water solubility, it causes a decrease in the tensile strength of the whey protein-pullulan-based film (Khanzadi *et al.,* 2015). The addition of wax is also reported to decrease WVP but increases the opacity of the chitosan coating (Rodrigues *et al.,* 2014). Moreover, wax also causes the surface structure of the coating to be inhomogeneous (Fabra *et al.,* 2009; Kowalczyk and Baraniak, 2014).

In other research, WVP is not only influenced by wax but also affected by oleic acid. Oleic acid increases the gloss effect and affects the particle size of the lipids (Vargas *et al.,* 2009). WVP is significantly affected by lipid particle size, where WVP efficiency increases with the smaller lipid particle size and the more homogeneous dispersion of hydrophobic materials (Pérez-Gago and Krochta, 2001). The instability phenomenon of large droplets of lipids in the composite film prepared from beeswax and oleic acid has been observed by Fabra *et al.* (2009) and Monedero *et al.* (2009) The film is opaque with a rough surface, which is associated with the accumulation of lipid aggregates on the surface of the film due to the coalescence and cream formation during film drying. The stability of lipid-based coating can be improved by applying nanotechnology approaches through the formation of small lipid droplets.

Nanoemulsion is an emulsion system with a very small droplet size, ranging from 20 to 200 nm or up to 500 nm (Tadros *et al.,* 2004). Kinetically, nanoemulsion is stable because of its tiny size and prevents creaming and sedimentation (Acevedo-fani *et al.,* 2017). In addition, with petite particle size, the nanoemulsion appears transparent (Wakisaka *et al.,* 2014), which is beneficial for fruit coating purposes. In the manufacture of nanoemulsions, the formulation/composition of the emulsion will determine the particle size of the resulting nanoemulsion. e Silva *et al.* (2017) mentioned that the appropriate particle size could be obtained by optimizing the proportion of the emulsifier. Lipid volume and viscosity also affect the formation of nanoscale particles. According to Shariffa *et al.* (2016), the suitable emulsifier effectively reduces the interfacial tension between the dispersed phase and the dispersing phase to affect the droplets during the homogenization process. The emulsifier forms interfacial layers on the droplets, which prevent the droplets from aggregating. In this study, Tween 80, a surfactant with an HLB value of 15, was used to stabilize the oil-in-water emulsion system. This research aimed to examine the effect of the ratio of beeswax and oleic acid and the concentration of Tween 80 on the physical characteristics of the lipid nanoemulsion.

2. Materials and methods

2.1 Materials

Beeswax (purchased from a local producer in Surabaya, Indonesia) and oleic acid 99% (PT Musim Mas, Medan-Indonesia) were used as the lipid phase. Glycerol 99.5% (PT. Sumi Asih Oleochemical Industry, Indonesia) was used as a plasticizer agent in the formulation. Tween 80 (PT. KAO Indonesia Chemicals) as a surfactant and distilled water was used as a continuous phase.

2.2 Preparation of lipids nanoemulsion

Nanoemulsions were prepared using a modified procedure proposed by e Silva *et al.* (2017). A 5% (w/w) of lipid fraction consisting of beeswax (BW) and oleic acid (OA) at different ratios (100:0, 75:25, 50:50, and 25:75), 1% glycerol w/w, different concentrations of Tween 80 (2.5%, 5%, 7.5%, 100%) as surfactant and water as aqueous phase was heated separately to 75-80°C. The molten BW was mixed with the heated oleic acid, and then the surfactant was added into the lipid phase while continuing to stir with a magnetic stirrer. In a separate container, the hot water and glycerol were mixed and then added into the lipid solution while stirring for 2 mins. Subsequently, the solution was homogenized for 5 mins at 8,000 rpm using a high-shear homogenizer (Silverson L4R, England) to form a nanoemulsion. The resulting nanoemulsions were placed in 15 mL tubes for further analysis.

2.3 Determination of pH

The pH value was determined using a digital pH meter (Laqua-PH 1100, Horiba Ltd, Japan). The instrument was calibrated using a buffer at pH 4 and 7 before use. The pH was measured by dipping the electrode probe in the lipid nanoemulsion. The readings were taken when the pH meter showed a stable number.

2.4 Determination of viscosity

The viscosity of nanoemulsions was measured using a Brookfield viscometer (USA equipped with 5 spindles of different sizes. Nanoemulsions with low viscosity were measured using spindle 1 at a rotating speed of 60 rpm, while those with high viscosity were measured using spindle 3 at a speed of 30 rpm. The selected spindle was immersed into the nanoemulsion and placed in a beaker glass (200 mL) up to the marker limit in the shaft. The viscometer was turned on and the readings were taken after 2 mins. Values were expressed in cP (mPa.s).

2.5 Determination of particle size, polydispersity index and zeta-potential

The droplet size of nanoemulsions was measured by dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instrument Ltd, UK). The instrument worked at 633 nm at 25°C and was equipped with a backscatter detector (173^o). Average droplet size and PDI were used to characterize droplet dispersion in nanoemulsion based on the Brownian motion of the particle in solution. Droplet diameter and PDI value of nanoemulsion were measured at 10-fold, 100-fold, and 1000-fold water dilutions. A PDI near 1 indicates a heterogeneous distribution between droplet sizes. The surface charge (*ζ-potential*) of nanoemulsion droplets was determined by measuring the electrophoretic flexibility at 25°C, and zeta-potential values were expressed in mV. Each sample was evaluated at least three times.

2.6 Determination of nanoemulsion stability using freeze-thaw method

The physical stability of lipid nanoemulsions was determined by the freeze-thaw method (Gozali *et al.* 2009). The lipid nanoemulsion was placed in the vial (t = 5.2 cm, d = 2 cm), then the samples were stored in the refrigerator at 4°C for 24 hrs and then transferred to the oven at 40°C for 24 hrs (calculated as one cycle). The test was carried out for seven cycles. The phase separation was measured using a micrometer in each cycle with the equation:

$$\%\ separation = \frac{the\ heigh\ of separation}{the\ heigh\ of\ total\ sample} \times 100$$

2.7 Statistical analysis

All the experiments were carried out in three replications. Statistical analysis was performed by one-way analysis of variance (ANOVA) using SAS version 9.2. Duncan's multiple range test (DMRT) with a 95% confidence level ($p < 0.05$) was considered statistically significant.

3. Results and discussion

3.1 The pH of nanoemulsion

Determination of pH is important because variation in pH influences the stability of nanoemulsions (Shu *et al.,* 2018). The pH of nanoemulsions ranged between 4.40 and 6.11 (Table 1). The statistical analysis showed that the pH of nanoemulsions was influenced by the lipid ratio and Tween 80 concentration. The incorporation of OA resulted in a decrease in the pH of the nanoemulsion. The decrease in pH of BW-OA nanoemulsion could be due to the presence of a carboxylate group of oleic acid (Vargas *et al.,* 2009). Increasing the concentration of Tween 80 also had an effect on the pH of the nanoemulsion. At a higher concentration, Tween 80 produced nanoemulsions with higher pH values. This was presumably due to the contribution of pH of Tween 80 which was in the range of 6-8 (Rowe *et al.,* 2009). The changes in the pH could suggest the existence of free fatty acids in the nanoemulsion (e Silva *et al*., 2017).

3.2 Viscosity

Viscosity is an important parameter determining the surface properties of the coating. Viscous emulsion would produce a thick and uneven coating on the fruit surface. The effects of the BW:OA ratio and Tween 80 concentration on the viscosity of nanoemulsion are shown in Table 2. The viscosity of nanoemulsion ranged between 3.13 cP and 520.0 cP. The statistical analysis showed that the viscosity of nanoemulsions was influenced by the lipid ratio and Tween 80 concentration. When the number of OA was equal to or greater than BW, an increase in concentration of Tween 80 increased the viscosity. On the other hand, when the amount of OA was smaller than OA, the increase in Tween 80 did not affect on viscosity of the nanoemulsion. The increase in viscosity could be due to the increase in the amount of emulsifiers that increased the total dissolved solids. The same result was also observed by Yuliasari *et al.* (2014) that increasing the concentration of Tween 80 led to an increase in viscosity due to an increase in the total solids in the nanoemulsion. According to McClements (2007) and Pal (2011), the viscosity of the emulsion was

Table 1. The pH of nanoemulsion of different lipid ratios and Tween 80 concentrations.

Lipid Ratio	Tween 80 (%)			
(BW:OA)	2.5	5	7.5	10
100:00:00	5.25±0.00^{Ba}	5.39±0.20^{Ba}	6.04±0.00^{Cb}	6.11±0.01^{Bb}
75:25:00	4.56±0.11^{Aa}	5.17 ±0.11^{Bb}	5.06±0.35^{Bb}	5.11±0.35^{Ab}
50:50:00	4.46±0.03^{Aa}	4.44±0.06^{Aa}	4.73±0.06^{Ab}	4.76±0.06^{Ab}
25 : 75	4.46±0.04^{Aa}	4.40±0.04^{Aa}	4.51±0.00^{Aa}	4.93±0.05^{Ab}

Values are presented as mean±SD (n = 3). Values with different uppercase superscripts within the same column are statistically significantly different due to lipid ratio (p<0.05) as determined by Duncan's Multiple Range Test (DMRT). Values with different lowercase superscripts within the same row are statistically significantly different among Tween 80 concentrations (p<0.05) as determined by Duncan's Multiple Range Test (DMRT).

Table 2. The viscosity (centipoise, cP) of nanoemulsion of different lipid ratios and Tween 80 concentrations.

Lipid Ratio	Tween 80 (%)			
(BW:OA)	2.5	5	7.5	10
100:00:00	3.13±0.18^{Aa}	3.25±0.71^{Aa}	4.63±0.53^{Aa}	5.00±0.00^{Aa}
75:25:00	3.25±0.35^{Aa}	2.75±0.35^{Aa}	3.00±0.00^{Aa}	4.13±0.18^{Aa}
50:50:00	3.13±0.18^{Aa}	4.88±0.18^{Aa}	265.00±21.21^{Bb}	315.00±21.21^{Bc}
25 : 75	3.13±0.53^{Aa}	15.50±0.71^{Aa}	285.00±21.21^{Bb}	520.00±21.21^{Cc}

Values are presented as mean±SD (n = 3). Values with different uppercase superscripts within the same column are statistically significantly different due to lipid ratio (p<0.05) as determined by Duncan's Multiple Range Test (DMRT). Values with different lowercase superscripts within the same row are statistically significantly different among Tween 80 concentrations (p<0.05) as determined by Duncan's Multiple Range Test (DMRT).

influenced by several factors, including the concentration of the dispersed phase, the ratio of the dispersed and emulsifying phase, and the particle size. In this study, the results showed that the viscosity was closely associated with the particle sizes of nanoemulsions, where at a higher concentration of OA, the addition of Tween 80 (7.5% and 10%) resulted in nanoemulsions with larger sizes. According to Khorram *et al.* (2017), the solid content and viscosity of the coating affected of coating thickness, internal gas modification of coated fruit and respiration rate of the fruit during storage.

3.3 Particle size

The particle size ranged between 18.02 nm and 457 nm (Figure 1). Particle sizes of nanoemulsion were significantly affected by both the BW:OA ratio and the concentration of Tween 80. The particle size with BW:OA ratio of 100:0 and 75:25 decreased with increasing Tween 80 concentration. Increasing the concentration of Tween 80 resulted in a decrease in the particle size of the nanoemulsion at the dispersed phase of 100 % BW. A more significant decrease in particle size due to an increase in Tween 80 concentration occurred in nanoemulsion with BW:OA ratio of 75:25. The smallest particle size was obtained by incorporating 10% of Tween 80. The use of suitable surfactants determines the success of nanoemulsion formation. Surfactants play a role in droplet formation by lowering the interfacial tension, which reduces resistance to droplet deformation (Vargas *et al*., 2009; Gundewadi *et al*., 2018). Surfactants are adsorbed quickly on the surface of the oil phase, thereby effectively reducing the interfacial tension between the oil and water phases (Shu *et al*., 2018). The same results were also observed by Jadhav *et al.* (2015), that the average particle diameter of paraffin wax nanoemulsion decreased with increasing surfactant concentration. A higher concentration of surfactant enhances the interfacial area and reduces interfacial tension which causes a reduction in particle size. Surfactants also reduce the flocculation effect in the process of nanoemulsion instability because surfactants are able to provide better steric stability against flocculation (McClements, 2007).

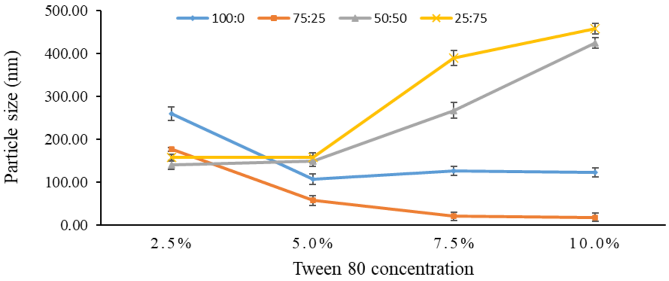

Figure 1. Effects of lipid ratio (BW:OA) and Tween 80 concentration on the particle size of lipid nanoemulsion. Each point corresponds to the average obtained from three replications.

On the contrary, an increase in the concentration of Tween 80 actually caused an increase in particle size when the addition of oleic acid in an equal amount to or greater than the BW (Figure 1). The tendency of increasing particle size with increasing concentration of Tween 80 at a high proportion of OA may be related to the role of OA as a surfactant. The presence of excess surfactant could induce the formation of micellar aggregates which increase the size of the oil droplet. A similar result was reported by Vargas *et al.* (2009), where more oleic acid could increase the particle size of chitosan-oleic acid film-forming dispersions. According to Rhim and Shelllhammer (2005), in the emulsion system, fatty acids act as a dispersed agent and also as a surfactant. So, the increasing number of oleic acid leads to an increase in the amount of surfactant in the nanoemulsion system. Flores *et al.* (2016) reported that the use of excess surfactant resulted in larger particle sizes due to the formation of micellar clusters in the emulsion system. Vaidya and Ganguli (2019) explained that excess surfactant causes the surface of oil droplets to become saturated so that surfactants begin to aggregate and form micelles. This point of concentration is called the critical micelle concentration.

3.4 Polydispersity index

The polydispersity index (PDI) is a parameter to define the particle size distribution of the nanoemulsion system. PDI measures the uniformity of the sample based on the particle size of the nanoemulsion. From the study results, the PDI values ranged from 0.164 to 0.610

(Figure 2). This value was the PDI medium value, in the range of 0.05-0.7 (Malvern Instruments Worldwide, 2011). These values indicate a homogeneous distribution of the resulting lipid nanoemulsion system. The PDI value of >0.7 indicates that the sample has an extensive particle size distribution and tends to be prone to sedimentation (Danaei *et al.,* 2018). In this study, the PDI value was significantly affected by the lipid ratio and the concentration of Tween 80. Generally, the change in PDI values found in this study had the same tendency as the particle size of the nanoemulsion. An enhancement of Tween 80 concentration led to a decrease in the PDI value of the nanoemulsion system on the addition of a lipid mixture with an amount of BW greater than OA. Conversely, increasing the concentration of Tween 80 increased the PDI value at an amount of OA equal to or greater than BW. The PDI value increased along with the increase in nanoemulsion particle size. The increase in PDI value could be due to an increasing amount of surfactant of Tween 80 and oleic acid that form micelle (Vargas *et al.,* 2009; Marhamati *et al.,* 2021). The higher PDI values of lipid nanoemulsion indicate a wide particle size variation. In addition to the larger particle size and PDI value of nanoemulsions, excess surfactant also resulted in nanoemulsions with high viscosity levels (Table 2). Therefore, an increase in the PDI value indicates a more considerable droplet size variation. The same results were also found in a study by Arnon-Rips *et al.* (2019), where the PDI value and particle size in sunflower and citral oil nanoemulsions increased with increasing Tween 80 concentrations.

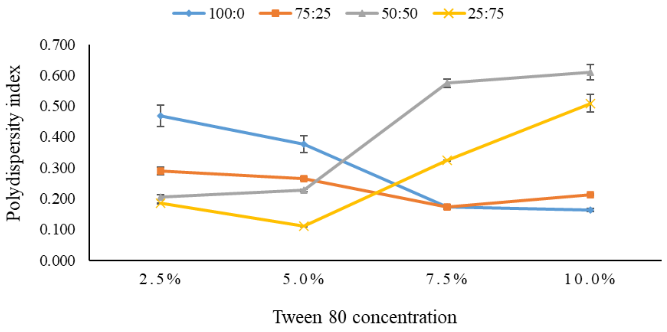

Figure 2. Effects of lipid ratio (BW:OA) and Tween 80 concentration on the polydispersity index (PDI) of lipid nanoemulsion. Each point corresponds to the average obtained from three replications.

3.5 Zeta-potential

The zeta-potential value of the lipid nanoemulsion mixture of beeswax and oleic acid in this study ranged from -9.71 to -38.35 mV (Figure 3). The ζ-potential of nanoemulsion was influenced by the ratio of BW:OA and the concentration of Tween 80. At the BW:OA ratio of 100:0 and 75:25, the negative charge of the zeta-potential tended to decrease with the addition of the Tween 80, which was from -24.95 mV to -19.50 mV and -22.55 mV to -12.60 mV, respectively. In general food emulsion systems, the droplets have an electric charge due to the adsorption of molecules on their surface. The charge present depends on the type and concentration of the ionized charge and the physical properties of the surrounding liquid (McClements, 2007). Determination of the electrical properties of the droplets in the emulsion system can be done by measuring the zeta-potential (ζ-potential). This value is commonly used to predict the surface charge which can be employed for understanding the physical stability of a colloidal system (Teo *et al.,* 2015). From this study, the zeta-potential value of the lipid mixture nanoemulsion in all samples was negative (Figure 3). This negative charge might be related to the charge on the carboxyl group of beeswax and oleic acid. According to Fratini *et al.* (2016), the main components of beeswax are free fatty acids such as palmitic, palmitoleic, and long-chain oleic esters of aliphatic alcohols. The lower zeta-potential values were allegedly due to the use of Tween 80 in large numbers. Since Tween 80 is a nonionic surfactant, the negative charges of nanoemulsion were dominated by BW. These results are in agreement with the research of e Silva *et al.* (2017), which reported that the zeta-potential value of the carnauba wax emulsion produced is low due to the use of surfactants Tween 80 with high concentrations. Another study by Anarjan and Tan (2013) using various polysorbate surfactants also resulted in a low negative zeta-potential charge.

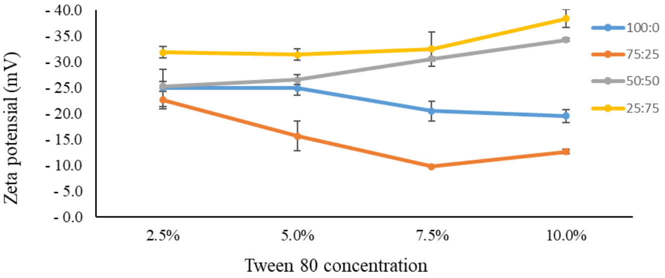

Figure 3. Effects of lipid ratio (BW:OA) and Tween 80 concentration on the zeta-potential of nanoemulsion. Each point corresponds to the average obtained from three replications.

On the other hand, at the BW:OA ratio of 50:50 and 25:75, the zeta-potential negative charge of the nanoemulsion increased with increasing concentration of Tween 80. The results were presumably due to the addition of oleic acid in higher quantities increased the presence of surface ions on the particles. Vargas *et al.* (2009) reported that zeta-potential of a dispersion made with Tween 80 and oleic acid possesses negatively charged particles with two types of particles, oleic acid micelles and oleic acid -Tween 80 micelles.

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The emulsion stabilization mechanism by the emulsifier consists of electrostatic and steric mechanisms. McClements (2007) noted a high zeta-potential value indicates an emulsion was stable to separation as a result of electrostatic repulsion. In general, charge stabilization of nanoemulsions was considered adequate when the zeta-potential was above ~30 mV. However, other groups of researchers (Mcclements, 2007; Zhang *et al*., 2008) also explained that non-ionic emulsifiers (including Tween 80) do not affect the electrostatic stability of emulsions, but are excellent steric stabilizers.

3.6 Freeze-thaw stability

The physical stability of the nanoemulsion was measured by the Freeze-thaw method. The observations for seven cycles of freeze-thaw can be seen in Figure 4. The stability of the 16 sample formulas showed different results (Figure 4). Some samples (L1T4, L2T1, L2T2, L2T3, L2T4, L3T1, L3T2, L4T2, L4T3) remained stable during the seven testing cycles. The emulsions were considered stable if there was no phase separation from the beginning of the nanoemulsion formation until the seventh cycle. This stability was strongly influenced by particle size. Lipid nanoemulsions with small particle sizes were more stable than those with larger sizes. In addition, the stable sample also had a PDI value of less than 0.5, which ranges from 0.111-0.325. The PDI values showed that the nanoemulsion system had high stability. The study is in accordance with the statement put forward by various research groups (Fryd and Mason, 2012; Silva *et al*., 2017; Gundewadi *et al*., 2018).

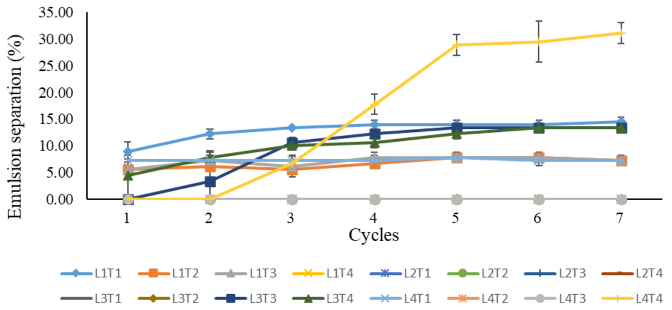

Figure 4. Effects of lipid ratio (BW:OA) and Tween 80 concentration on the stability of lipid nanoemulsion. Each point corresponds to the average obtained from three replications. L = BW:OA, L1 = 100:0, L2 = 75:25, L3 = 50:50, L4 = 75:25; T = Tween 80; T1 = 2.5%, T2 = 5.0%, T3 = 7.5%, T4 = 10%.

By looking at the results of the stability test and also considering the particle size, for further research, the selected nanoemulsion was a lipid mixture nanoemulsion with the L2T3 formula, with was BW:OA ratio of 75:25 and a Tween 80 concentration of 7.5%. The formula produced a lipid nanoemulsion with a particle size of 20.34 nm, PDI 0.173, zeta-potential -9.17, and a viscosity level of 3.00 cP. The nanoemulsion particle size of the selected formula was in accordance with the findings of Fryd and Mason (2012); the nanoemulsion has a particle size of less than 100 nm. Furthermore, a small PDI value (PDI<0.5) indicates a narrow particle size distribution indicating the homogeneity of the nanoemulsion produced. Both of these were crucial factors to obtain nanoemulsions with good stability (e Silva *et al*., 2017; Gundewadi *et al.,* 2018). Moreover, the low zeta-potential negative charge of this formula (-9.17 mV) indicated that the stabilization mechanism of the nanoemulsion was steric hindrance. This was in accordance with the study of Anarjan and Tan (2013), that the primary mechanism of nanoemulsion stabilizing nonionic emulsifiers such as polysorbates and sucrose esters was the steric effect.

4. Conclusion

Lipid nanoemulsion with particle size less than 500 nm was obtained from a mixture of beeswax and oleic acid as the dispersed phase and water as the dispersing phase. The use of Tween 80 as a surfactant effectively reduced the particle size. However, the higher oleic acid ratio of beeswax increased the particle size while also increasing the viscosity of the emulsion system. The decrease in nanoemulsion particle size was accompanied by an increase in the concentration of Tween 80 at BW:OA ratios of 100:0 and 75:25, which have the same tendency as the PDI value. Low PDI value indicated the nanoemulsion was monodispers or homogeneous, and had good stability. The small zeta-potential value in this study indicated that the stability of the nanoemulsion occurred through a steric mechanism. The results show the best formulation was BW:OA ratio of 75:25 at a Tween 80 concentration of 7.5 %. From this study, it was found that the formation of stable nanoemulsions was strongly influenced by the composition of the constituent materials, both the dispersed and dispersing phases as well as additives components such as surfactants.

Conflict of interest

The authors delcare no conflict of interest.

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References

Acevedo-Fani, A., Soliva-Fortuny, R. and Martín-Belloso, O. (2017). Nanoemulsions as edible coatings. *Current Opinion in Food Science*, 15, 43-49. https://doi.org/10.1016/j.cofs.2017.06.002

Alali, A.A., Awad, M.A., Al-qurashi, A.D. and Mohamed, S.A. (2018). Postharvest gum Arabic and salicylic acid dipping affect quality and biochemical changes of ‘Grand Nain’ bananas during shelf life. *Scientia Horticulturae*, 237(11), 51-58. https://doi.org/10.1016/j.scienta.2018.03.061

Anarjan, N. and Tan, C.P. (2013). Effects of selected polysorbate and sucrose ester emulsifiers on the physicochemical properties of astaxanthin nanodispersions. *Molecules*, 18(1), 768-777. https://doi.org/10.3390/molecules18010768

Arnon, H., Zaitsev, Y., Porat, R. and Poverenov, E. (2014). Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. *Postharvest Biology and Technology*, 87(1), 21-26. https://doi.org/10.1016/j.postharvbio.2013.08.007

Arnon-Rips, H., Porat, R. and Poverenov, E. (2019). Enhancement of agricultural produce quality and storability using citral-based edible coatings; the valuable effect of nano-emulsification in a solid-state delivery on fresh-cut melons model. *Food Chemistry*, 277(8), 205-212. https://doi.org/10.1016/j.foodchem.2018.10.117

Danaei, M., Dehghankhold, M., Ataei, S., Davarani, F.H., Javanmard, R., Dokhani, A., Khorasani, S. and Mozafari, M.R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*, 10(2), 57. https://doi.org/10.3390/pharmaceutics10020057

Fabra, M.J., Talens, P. and Chiralt, A. (2009). Microstructure and optical properties of sodium caseinate films containing oleic acid-beeswax mixtures. *Food Hydrocolloids*, 23(3), 676-683. https://doi.org/10.1016/j.foodhyd.2008.04.015

Flores, Z., San Martín, D., Villalobos-Carvajal, R., Tabilo-Munizaga, G., Osorio, F. and Leiva-Vega, J. (2016). Physicochemical characterization of chitosan-based coating-forming emulsions: Effect of homogenization method and carvacrol content. *Food Hydrocolloids*, 61(11), 851-857. https://doi.org/10.1016/j.foodhyd.2016.07.007

Fratini, F., Cilia, G., Turchi, B. and Felicioli, A. (2016). Beeswax: A minireview of its antimicrobial activity and its application in medicine. *Asian Pacific Journal of Tropical Medicine*, 9(9), 839-843. https://doi.org/10.1016/j.apjtm.2016.07.003

Fryd, M.M. and Mason, T.G. (2012). Advanced nanoemulsions. *The Annual Review of Physical Chemistry*, 63(22), 493-518. https://doi.org/10.1146/annurev-physchem-032210-103436

Gozali, D., Rusmiati, D. and Utama, P. (2009). Formulasi dan uji stabilitas mikroemulsi ketokonazol sebagai antijamur *Candida albicans* dan *Tricophyton mentagrophytes*. *Farmaka*, 7(2), 54-67. [In Bahasa Indonesia].

Gundewadi, G., Rudra, S.G., Sarkar, D.J. and Singh, D. (2018). Nanoemulsion based alginate organic coating for shelf life extension of okra. *Food Packaging and Shelf Life*, 18(4), 1-12. https://doi.org/10.1016/j.fpsl.2018.08.002

Hassan, B., Chatha, S.A.S., Hussain, A.I., Zia, K.M. and Akhtar, N. (2018). Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *International Journal of Biological Macromolecules*, 109(5), 1095-1107. https://doi.org/10.1016/j.ijbiomac.2017.11.097

Jadhav, A.J., Holkar, C.R., Karekar, S.E., Pinjari, D.V. and Pandit, A.B. (2015). Ultrasound Assisted Manufacturing of Paraffin Wax Nanoemulsions: Process Optimization. *Ultrasonics Sonochemistry.* 23(3), 201-207. https://doi.org/10.1016/j.ultsonch.2014.10.024

Joshi, A.V. and Rao, T.V.R. (2018). Edible coating delay ripening and influence the quality and shelf life of fruits and vegetable. *The Journal of Plant Science Research*, 34(1), 1-7.

Khanzadi, M., Mahdi, S., Mirzaei, H., Khodaian, F., Maghsoudlou, Y. and Dehnad, D. (2015). Physical and mechanical properties in biodegradable films of whey protein concentrate – pullulan by application of beeswax. *Carbohydrate Polymers*, 118(4), 24-29. https://doi.org/10.1016/j.carbpol.2014.11.015

Khorram, F., Ramezanian, A. and Hosseini, S.M.H. (2017). Shellac, gelatin and Persian gum as alternative coating for orange fruit. *Scientia Horticulturae*, 225(12), 22-28. https://doi.org/10.1016/j.scienta.2017.06.045

Klangmuang, P. and Sothornvit, R. (2016). Combination of beeswax and nanoclay on barriers , sorption isotherm and mechanical properties of hydroxypropyl methylcellulose-based composite films. *LWT - Food Science and Technology*, 65(1), 222-227. https://doi.org/10.1016/j.lwt.2015.08.003

Kowalczyk, D. and Baraniak, B. (2014). Effect of candelilla wax on functional properties of biopolymer emulsion films: A comparative study. *Food Hydrocolloid*s, 41(8), 195-209. https://doi.org/10.1016/j.foodhyd.2014.04.004

Malvern Instruments Worldwide. (2011). Dynamic Light Scattering: Common Terms Defined. Inform White Paper. Retrieved from website: https://facilities.bioc.cam.ac.uk/files/media/dls_terms_defined_malvern.pdf

Marhamati, M., Ranjbar, G. and Rezaie, M. (2021). Effects of emulsifiers on the physicochemical stability of oil-in-water nanoemulsions : A critical review. *Journal of Molecular Liquids*, 340, 117218. https://doi.org/10.1016/j.molliq.2021.117218

Marpaung, M., Ahmad, U. and Suyatma, N.E. (2015). Pelapis nanokomposit untuk pengawetan salak pondoh terolah minimal. *Jurnal Keteknikan Pertanian*, 3(1), 73-80. https://doi.org/10.19028/jtep.03.1.73-80 [In Bahasa Indonesia].

McClements, D.J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition*, 47(7), 611-649. https://doi.org/10.1080/10408390701289292

Monedero, F.M., Fabra, M.J., Talens, P. and Chiralt, A. (2009). Effect of oleic acid-beeswax mixtures on mechanical, optical and water barrier properties of soy protein isolate based films. *Journal of Food Engineering*, 91(4), 509-515. https://doi.org/10.1016/j.jfoodeng.2008.09.034

Nor Amalini, A., Norziah, M.H., Khan, I. and Haafiz, M.K.M. (2018). Exploring the properties of modified fish gelatin films incorporated with different fatty acid sucrose esters. *Food Packaging and Shelf Life*, 15(1), 105-112. https://doi.org/10.1016/j.fpsl.2017.12.003

Pal, R. (2011). Influence of interfacial rheology on the viscosity of concentrated emulsions. *Journal of Colloid And Interface Science*, 356(1), 118-122. https://doi.org/10.1016/j.jcis.2010.12.068

Pérez-Gago, M.B. and Krochta, J.M. (2001). Lipid particle size effect on water vapor permeability and mechanical properties of whey protein/beeswax emulsion films. *Journal of Agricultural and Food Chemistry*, 49(2), 996-1002. https://doi.org/10.1021/jf000615f

Rhim, J.W. and Shelllhammer, T.H. (2005). Lipid-Based Edible Films and Coatings. In Han, J.H. (Ed.). *Innovation in Food Packaging,* p. 362-383. Plano, TX, USA: Elsevier Ltd. https://doi.org/10.1016/B978-012311632-1/50053-X

Rodrigues, D.C., Caceres, C.A., Ribeiro, H.L., Abreu, R.F.A., Cunha, A.P. and Azeredo, H.M.C. (2014). Influence of cassava starch and carnauba wax on physical properties of cashew tree gum-based films. *Food Hydrocolloids*, 38(5), 147-151. https://doi.org/10.1016/j.foodhyd.2013.12.010

Rowe, R.C., Sheskey, P.J. and Quinn, M.E. (2009). Handbook of Pharmaceutical Excipients. 6th ed. Washington DC, USA: Pharmaceutical Press.

Shariffa, Y.N., Tan, T.B., Abas, F., Mirhosseini, H., Nehdi, I.A. and Tan, C.P. (2016). Producing a lycopene nanodispersion: The effects of emulsifiers. *Food and Bioproducts Processing*, 98(2), 210-216. https://doi.org/10.1016/j.fbp.2016.01.014

Shu, G., Khalid, N., Chen, Z., Neves, M.A., Barrow, C.J. and Nakajima, M. (2018). Formulation and characterization of astaxanthin-enriched nanoemulsions stabilized using ginseng saponins as natural emulsifiers. *Food Chemistry*, 255(18), 67-74. https://doi.org/10.1016/j.foodchem.2018.02.062

Silva, P.C., Pereira, L.A.S., Carvalho, G.R., Campelo, P.H., Botrel, D.A., Oliveira, J.E. and Marconcini, J.M. (2017). Production and stability of carnauba wax nanoemulsion. *Advanced Science, Engineering and Medicine*, 9(11), 977-985. https://doi.org/10.1166/asem.2017.2072

Sothornvit, R. and Krochta, J.M. (2001). Plasticizer effect on mechanical properties of beta-lactoglobulin film. *Journal of Food Engineering*, 50(3), 149-155. https://doi.org/10.1016/S0260-8774(00)00237-5

Tadros, T., Izquierdo, P., Esquena, J. and Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 109(1), 303-318. https://doi.org/10.1016/j.cis.2003.10.023

Teo, S.Y., Lee, S.Y., Ong, H.L., Ong, C.L., Gan, S.N., Rathbone, M.J. and Coombes, A.G.A. (2015). Evaluation of biosourced alkyd nanoemulsions as drug carriers. *Journal of Nanomaterials*, 2015, 537598. https://doi.org/10.1155/2015/537598

Thakur, R., Pristijono, P., Bowyer, M., Singh, S.P., Scarlett, C.J., Stathopoulos, C.E. and Vuong, Q.V. (2019). A starch edible surface coating delays banana fruit ripening. *LWT - Food Science and Technology*, 100(2), 341-347. https://doi.org/10.1016/j.lwt.2018.10.055

Vaidya, S., and Ganguli, A.K. (2019). 2.01 - Microemulsion methods for synthesis of nanostructured materials. In Andrews, D., Nann, T. and Lipson, R. (Eds.), Comprehensive Nanoscience and Nanotechnology. Vol. 2, 2nd ed., p. 1-12. USA: Academic Press. https://doi.org/10.1016/B978-0-12-803581-8.11321-9

Vargas, M., Albors, A., Chiralt, A. and Gonzalez-Martınez, C. (2009). Characterization of chitosan - oleic acid composite films. *Food Hydrocolloids*, 23 (3), 536-547. https://doi.org/10.1016/j.foodhyd.2008.02.009

Vargas, M., Pastor, C., Chiralt, A., McClements, D.J. and González-Martínez, C. (2008). Recent advances in edible coatings for fresh and minimally processed fruits. *Critical Reviews in Food Science and Nutrition*, 48(6), 496-511. https://doi.org/10.1080/10408390701537344

Wakisaka, S., Nakanishi, M. and Gohtani, S. (2014). Phase behavior and formation of O/W nano-emulsion in vegetable oil/mixture of polyglycerol polyricinoleate and polyglycerin fatty acid ester/water systems. *Journal of Oleo Science*, 63(3), 229-237. https://doi.org/10.5650/jos.ess13139

Yousuf, B., Sha, O. and Kumar, A. (2018). Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: A review. L*WT - Food Science and Technology,* 89(3), 198-209. https://doi.org/10.1016/j.lwt.2017.10.051

Yuliasari, S., Fardiaz, D. and Andarwulan, N. (2014). Karakterisasi nanoemulsi minyak sawit merah yang diperkaya beta karoten. *Littri*, 20(3), 111-121. https://doi.org/10.21082/jlittri.v20n3.2014.111-121 [In Bahasa Indonesia].

Zhang, H., Feng, F., Li, J., Zhan, X., Wei, H., Li, H. and Wang, H. (2008). Formulation of food-grade microemulsions with glycerol monolaurate: effects of short-chain alcohols, polyols, salts and nonionic surfactants. *European Food Research and Technology*, 226(5), 613-619. https://doi.org/10.1007/s00217-007-0606-z

Zhang, Y., Simpson, B.K. and Dumont, M.J. (2018). Effect of beeswax and carnauba wax addition on properties of gelatin films: A comparative study. *Food Bioscience*, 26(6), 88-95. https://doi.org/10.1016/j.fbio.2018.09.011