AGROINDUSTRIAL TECHNOLOGY JOURNAL
ISSN : 2599-0799 (print) ISSN : 2598-9480 (online)
Accredited SINTA 3: No.225/E/KPT/2022

Stability and Characteristics of Red Palm Oil Nanoemulsions at Different Surfactant Ratios

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Article history:
Submitted: 29 January 2026
Revision: 16 March 2026
Accepted: 12 April 2026
Online: 31 May 2026
DOI: http://doi.org/10.21111/atj.v10i1.15

ABSTRACT: *β-carotene is a potent antioxidant naturally present in red palm oil (RPO) at concentrations of 500-700 ppm. Despite its significant nutritional and functional benefits, the application of β-carotene in aqueous food systems remains limited due to its hydrophobic nature and high sensitivity to heat, light, and oxygen. Therefore, the development of an effective lipid-based delivery system is required to improve its dispersibility and stability. Nanoemulsions, lipid-based carriers with nanosized droplets, have emerged as an effective approach for such applications. This study aimed to evaluate the stability and physicochemical characteristics of β-carotene nanoemulsion formulated using a red palm oil-palm oil blend and produced via a low-energy emulsification method (phase inversion). Tween 80 was used as the surfactant, and distilled water as the aqueous phase. The nanoemulsions were characterized based on turbidity, particle size distribution, polydispersity index (PDI), zeta potential, pH, viscosity, color attributes, and β-carotene retention during storage at room temperature. The results showed that the surfactant-to-oil ratio significantly influenced the physical stability of the nanoemulsions. The optimal formulation, achieved at a surfactant-to-oil ratio of 3:1, produced a nanoemulsion with an average particle size of approximately 200 nm, a low polydispersity index, and a stable zeta potential for up to four weeks. Chemical stability, as measured by β-carotene content, declined markedly after 3 weeks of storage. These findings indicate that blending red palm oil with palm oil enables the formation of physically stable nanoemulsions using a low-energy method. Nevertheless, further formulation optimization is required to enhance the chemical stability of β-carotene for prolonged storage and potential application in aqueous food systems.*

Keywords: *β-carotene; Nanoemulsions; Palm Oil; Red Palm Oil; Storage*

1. INTRODUCTION

Carotenoids are natural bioactive compounds widely recognized for their antioxidant activity and health-promoting effects. Among them, β-carotene is a provitamin A compound with strong antioxidant activity and is abundantly found in red palm oil (RPO) at concentrations of 500-700 ppm (Pignitter et al., 2016; Wahyuni & Sulistyani, 2021). In addition to β-carotene, RPO also contains other bioactive compounds such as tocopherols and tocotrienols, which contribute to its functional properties (Mba et al., 2015). Consequently, maintaining β-carotene stability during food processing and storage remains a major challenge due to its hydrophobicity and high susceptibility to degradation induced by heat, oxygen, and light (Kusbandari & Susanti, 2017). These limitations reduce its stability and bioavailability, particularly in aqueous-based food products.

The stability of bioactive compounds in formulated food products is highly dependent on the composition and processing conditions of the system (Pulungan et al., 2021). Therefore, to overcome these challenges, developing a lipid-based delivery system is essential to increase its solubility and improve its stability in aqueous systems. Among these systems, nanoemulsions have attracted considerable attention due to their small droplet size, which enhances kinetic stability, optical clarity, and protection of encapsulated bioactive compounds (Mehmood et al., 2021a). In addition, nanoemulsions and nanostructured lipid carrier have been reported to provide better stability, bioavailability, solubility, and oxidative stability of lipophilic compounds compared to conventional emulsions (Elianarni et al., 2023; López-Monterrubio et al., 2021; Mehmood et al., 2021a). Nanoemulsions can also function as carrier systems that protect bioactive compounds from degradation during storage, as reported for resveratrol (Davidov-Pardo & Mcclements, 2015). Nanoemulsions have been shown to improve the oxidative stability and functional performance of antioxidants in food systems (Y. Sari et al., 2022).

Despite these advantages, the formulation of nanoemulsions containing red palm oil (RPO) still presents several challenges. The relatively high viscosity of RPO may hinder droplet dispersion during emulsification and often requires high-energy processing methods, which are less suitable for heat and oxidative sensitive compounds such as β-carotene (Dewandari & Sofwan, 2022). In addition, the formulation parameters, including oil phase composition and surfactant concentration affect the physicochemical properties and stability of nanoemulsions (Akram et al., 2021; Ozturk & Mcclements, 2016). Therefore, optimization of both the oil phase and surfactant system is necessary to obtain a stable nanoemulsion.

One approach to overcome these limitations is to modify the oil phase composition. Blending red palm oil with a lower-viscosity of oil, such as palm oil, may reduce the overall viscosity of the oil phase and facilitate nanoemulsions formation using low-energy emulsification methods such as emulsion phase inversion. In parallel, the use of non-ionic surfactants such as Tween 80 has been widely used to effectively reduce interfacial tension and produce smaller droplet sizes, which may improve nanoemulsions stability (Jo & Kwon, 2014). This approach may allow the production of nanoemulsions with lower energy input while maintaining the functional properties of red palm oil.

Previous studies have mainly focused on nanoemulsions systems produced using high-energy methods or relatively high surfactant concentrations. However, studies investigating the combination of oil phase modification through red palm oil-palm oil blending and low-energy emulsification methods remain limited. In particular, the effect of surfactant-to-oil ratio on the physicochemical characteristics and stability of such systems has not been widely reported. Therefore, further study is needed to evaluate the potential of this approach for β-carotene delivery.

Therefore, this study aimed to evaluate the effect of surfactant-to-oil ratios on the physicochemical characteristics and stability of β-carotene nanoemulsions prepared from a red palm oil-palm oil blend using a low-energy emulsion phase inversion method.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study included red palm olein and palm oil from commercial market, Tween 80 (Merck, Germany) and β-carotene, distilled water; and chloroform (Merck, Germany). The equipment used included HORIBA sz-100 (nanoPartica series, HORIBA, Ltd.) located at the Pharmaceutical Technology Laboratory of Universitas Islam Indonesia Yogyakarta, Shimadzu UV-Vis spectrophotometer (UV-mini 1240 CE), Ohaus centrifuge, vortex (M37610-33, USA) and hotplate magnetic stirrer (AREC).

2.2. Nanoemulsion Preparation

The preparation of β-carotene nanoemulsions was conducted through four sequential mixing stages, following a low-energy emulsification approach (Y. P. Sari et al., 2020). First, red palm oil and palm oil were mixed using a magnetic stirrer at 800 rpm for 5 minutes at room temperature to obtain a homogeneous oil phase according to the formulation shown in Table 1. Second, nanoemulsions were produced using emulsion phase inversion method. Tween 80 was added to the oil phase according to the predetermined surfactant-to-oil ratio. The mixture was stirred at 1000 rpm for 30 minutes to ensure complete dispersion of the surfactant within the oil phase. Third, the aqueous phase (distilled water) was added dropwise to the oil–surfactant mixture using a pipette while stirring at 1000 rpm for 30 minutes, allowing gradual phase inversion and nanoemulsion formation. Finally, the resulting mixture was further stirred at 1200 rpm for 30 minutes to obtain a uniform and stable nanoemulsions. During this stage, the beaker was covered with plastic wrap or aluminum foil to prevent sample loss due to splashing.

2.3. Nanoemulsion characterization

All samples were diluted in distilled water prior to analysis to avoid multiple scattering effects. The turbidity of selected nanoemulsions was measured at 600 nm using UV-Vis Spectrophotometer (Zhong et al., 2017). Particle size, polidispersity index, and zeta-potential were analyzed by using Horiba SZ-100. The pH and viscosity of the samples were analyzed using Hanna Instruments (HI2000 Series) and viscometer (Brookfield), respectively. Colour was analyzed using a colorimeter (NH 310). β-carotene content in nanoemulsions were analyzed according to the method described by (López-Monterrubio et al., 2021).

2.4. Experimental Design and Data Analysis

All of the analysis were done at least in triplicate. The experimental factor was the surfactant-to-oil ratio (red palm oil and palm oil), with five levels: 1:1, 1.5:1, 2:1, 2.5:1, and 3:1. The treatment combinations are presented in Table 1. The data were analyzed by using one-way analysis of variance (ANOVA) (IBM SPSS 25). When significant differences were observed, Duncan's multiple range test (DMRT) was performed to compare means among treatments at a significance level of $p < 0,05$.

Table 1. Treatment combinations in nanoemulsions

S:O (v/v)	RPO (ml)	Palm oil (ml)	Tween 80 (ml)	Distilled water (ml)	Total (ml)
1:1	1.25	1.25	2.5	20	25
1.5:1	1	1	3	20	25
2:1	0.83	0.83	3.33	20	25
2.5:1	0.714	0.714	3.571	20	25
3:1	0.625	0.625	3.75	20	25

Note S:O means surfactant to oil ratio

3. RESULTS AND DISCUSSION

3.1. Turbidity

Turbidity analysis was conducted to evaluate the physical stability of β-carotene nanoemulsions during storage as affected by the surfactant-to-oil ratio (SOR). As shown in Figure 1, the appearance of the nanoemulsions became progressively slight clearer with increasing SOR. Samples prepared at higher SOR exhibited greater transparency, whereas those prepared at lower SOR appeared more turbid. This visual observation was consistent with the turbidity measurements presented in Figure 2. The turbidity values of all samples ranged from 150-380 cm^{-1} in the first week, depending on the formulatio. An increase in SOR resulted in a significant decrease in turbidity, indicating improved optical clarity of the nanoemulsions system.

The decrease in turbidity was associated with the formation of smaller droplet sizes at higher surfactant concentrations. Increasing the amount of Tween 80 reduced interfacial tension, facilitating more efficient droplet disruption during emulsification and leading to the formation of finer and more uniformly distributed droplets (Y. P. Sari et al., 2020; Zhang & Li, 2022). Smaller droplets scatter less light, leading to lower turbidity values. These findings were consistent with the particle size results presented in Section 3.2, where formulations with higher SOR produced droplet sizes (approximately 200 nm).

During storage, turbidity remained relatively stable in formulations with higher SOR (2.5:1 and 3:1), indicating good physical stability and resistance to destabilization phenomena such as coalescence and creaming. In contrast, formulations with lower SOR showed greater fluctuations in turbidity, suggesting a higher tendency for droplet aggregation. Overall, these results demonstrate that the surfactant-to-oil ratio plays a critical role in controlling droplet size, optical properties, and physical stability of β-carotene nanoemulsions. Higher SOR levels enhance nanoemulsion stability by promoting smaller droplet formation and reducing light scattering, thereby improving system clarity and homogeneity.

This confirmed that turbidity can be used as a useful indicator of nanoemulsion stability, although direct measurements of particle size remain necessary for a more complete characterization.

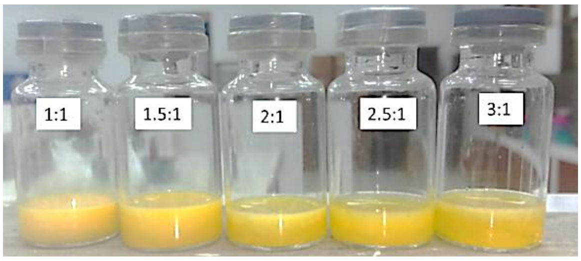

Figure 1. Appearance of nanoemulsion results from left to right SOR 1:1; 1.5:1; 2:1; 2.5:1 and 3:1 (w/w).

The presence of small droplets also influences the phase behavior and kinetic stability of nanoemulsions. Reduced droplet size can enhance resistance to gravitational separation and coalescence, thereby improving stability during storage (Mehmood et al., 2021b).

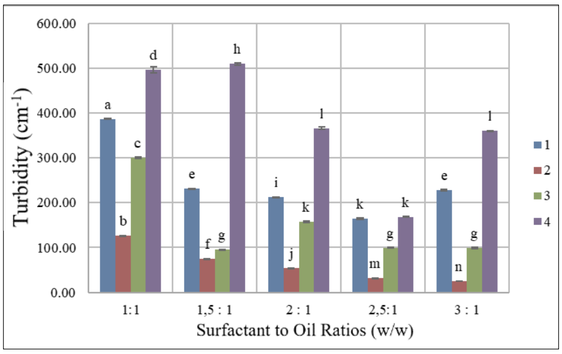

Figure 2. The effect of surfactant:oil ratio on the turbidity of nanoemulsion samples during storage for up to 4 weeks. The same superscript letter indicates no significant difference between samples (p>0.05).

3.2. Particle Size, PDI, and Zeta-Potential

Particle size analysis (z-average) demonstrated that the nanoemulsion formulated at a surfactant-to-oil ratio (SOR) of 3:1 achieved the smallest mean droplet size (around 200±20 nm) and remained stable for up to four weeks of storage (Figure 3a).

Highlighting the significance of formulation parameters, similar findings by Sari et al. (2020) showed that nanoemulsions prepared with a low-energy method and an SOR of 2.5:1 produced minimal droplet sizes when using a blend of palm oil and rice bran oil as the oil phase. The formation of nanoscale droplets enhances kinetic stability by reducing gravitational separation and droplet coalescence (Y. P. Sari et al., 2020). These results underscore that optimal nanoemulsion stability can be achieved at SORs of 2.5:1 and 3:1, even with low-energy emulsification techniques.

Previous work by Sari et al. (2018) demonstrated the successful use of Tween 80 in producing red palm oil nanoemulsions; however, a relatively high surfactant-to-oil ratio of 1:8 was employed. This study shows that comparable droplet sizes can be achieved with lower surfactant levels, emphasizing efficiency and potential cost savings for researchers and professionals (F. Sari et al., 2018). Similar to other surfactant-based liquid formulations, the stability and physicochemical properties of nanoemulsions are strongly affected by surfactant concentration and formulation design (Luketsi et al., 2022).

All nanoemulsion samples showed moderate polydispersity, with PDI values between 0.45 and 0.58 (Figure 3b). This size distribution is typical for low-energy prepared nanoemulsions, which tend to have broader droplet size ranges. The polydispersity index (PDI) values of the nanoemulsions ranged from 0.45 to 0.58, indicating a moderately broad droplet size distribution. In general, nanoemulsion systems with PDI values below 0.3 are considered to have a narrow, uniform size distribution, whereas higher values indicate greater heterogeneity in droplet size.

The relatively high PDI observed in this study is likely due to the use of a low-energy emulsification method, which typically produces a broader droplet size distribution than high-energy methods such as high-pressure homogenization. In low-energy systems, droplet formation is governed by spontaneous physicochemical processes, leading to less uniform droplet breakup. Despite moderate polydispersity, the nanoemulsions exhibited good physical stability during storage, as indicated by relatively stable turbidity values and consistent particle size measurements. This result suggests that the system was kinetically stable, even with a broader size distribution. The presence of sufficient surfactant (Tween 80) likely contributed to stabilizing droplets by forming an effective interfacial layer that prevented coalescence.

In addition, increasing the surfactant-to-oil ratio (SOR) tended to reduce droplet aggregation and improve size distribution. However, the PDI values remained above the threshold typically associated with highly monodisperse systems. This result indicates that while surfactant concentration improves homogeneity, the emulsification method remains a limiting factor in achieving lower PDI values. Overall, these findings suggested that the nanoemulsion system developed in this study exhibits acceptable stability for food applications. However, further optimization of formulation parameters or processing conditions may be required to achieve a more uniform droplet size distribution.

Zeta potential values ranged from −32 to −42 mV, indicating adequate electrostatic repulsion to prevent droplet aggregation and improve stability (Figure 3c). Despite Tween 80 being non-ionic, the negative zeta potential likely results from hydroxyl (OH^-) ions adsorbing onto droplet surfaces (Jo & Kwon, 2014; Y. P. Sari et al., 2020). The surface charge magnitude is crucial for colloidal stability, aligning with the consistent droplet size over four weeks. These findings confirmed that formulation composition directly influences zeta potential and nanoemulsion stability.

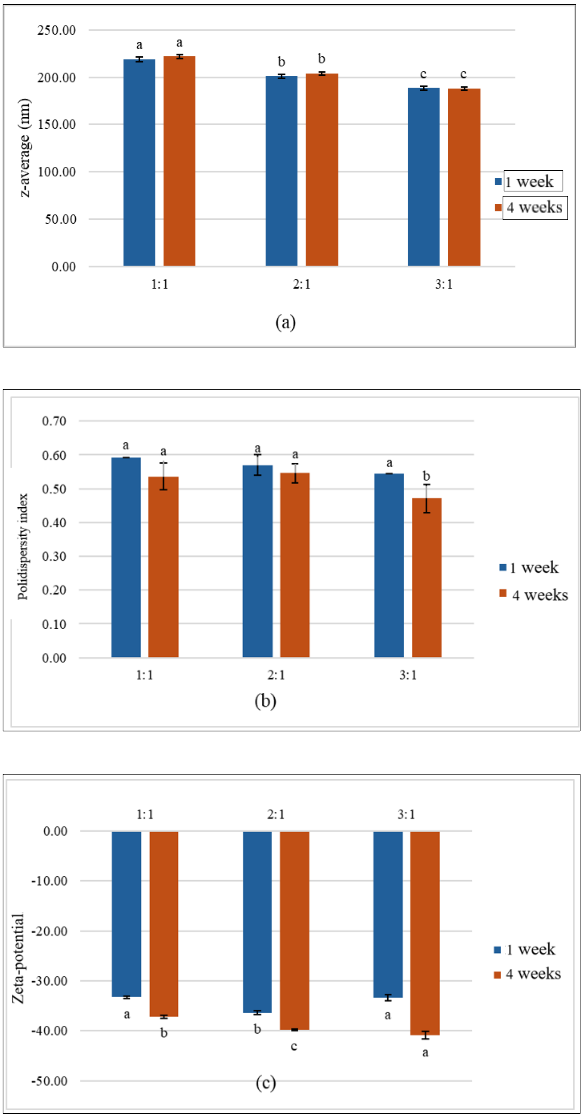

Figure 3. The effect of surfactant:oil ratio on size (z-average) (a), polydispersity index (b), and zeta-potential (c) of nanoemulsion samples during storage for up to 4 weeks. The same superscript letters indicate no significant differences between samples (p>0.05).

3.3. pH and Viscosity

The pH values of the nanoemulsions ranged from 5.44 to 5.59 (Table 2). These values were lower than those reported by Sari et al. (2018), who observed pH values of 6.3–7.0 in nanoemulsions formulated using red palm olein as the sole oil phase. A slight decrease in pH was observed with increasing surfactant-to-oil ratio (SOR); however, all formulations remained within a narrow and stable pH range, indicating good formulation stability. This behavior can be attributed to the use of Tween 80, a non-ionic surfactant that has minimal influence on hydrogen ion concentration and, consequently, does not cause significant pH fluctuations in the system (Jo & Kwon, 2014)**.**

A slight decrease in pH was observed with increasing surfactant-to-oil ratio (SOR), although all formulations remained within a relatively narrow range (5.44–5.59). While Tween 80 is a non-ionic surfactant that does not directly release or consume hydrogen ions, the observed pH reduction may be attributed to indirect effects arising from changes in the nanoemulsion system. One possible explanation is the increased interfacial area resulting from smaller droplet sizes at higher SOR. As surfactant concentration increases, more oil droplets form, increasing the total surface area and enhancing interaction between the dispersed and aqueous phases. This expanded interface may facilitate the partitioning or release of minor acidic components present in the oil phase into the aqueous phase, thereby slightly lowering the pH.

In addition, the higher concentration of surfactant molecules in the continuous phase may alter the system's microenvironment, including the orientation of water molecules and the interfacial structure, which can influence proton distribution and lead to minor pH shifts. Similar behavior has been reported in emulsion systems, where the intrinsic properties of the surfactant do not solely govern pH changes, but are also influenced by interfacial phenomena and system composition.

Despite this decrease, the pH values remained relatively stable across all formulations, indicating that the nanoemulsion system was not significantly affected in terms of chemical stability. This result suggests that Tween 80 is suitable for maintaining pH stability within the acceptable range for food applications.

Table 1. pH and viscosity values of nanoemulsion samples at various surfactant ratios: oil

Surfactant:Oil Ratio	pH	Viscosity
1:1	5.59 ± 0.01^{a}	19.23 ± 0.06^{a}
1.5:1	5.47 ± 0.02^{b}	22.43 ± 0.06^{b}
2:1	5.52 ± 0.03^{c}	22.1 ± 1.21^{b}
2.5:1	5.45 ± 0.01^{b}	25.27 ± 0.06^{c}
3:1	5.44 ± 0.01^{b}	26.4 ± 0.8^{c}

Note: Identical superscript letters at same column indicate no significant difference between the same columns at the 95% confidence level.

Viscosity increased significantly ($p < 0.05$) with increasing surfactant-to-oil ratio (SOR), ranging from 19.23 to 26.40 cP. This increase can be attributed to the higher concentration of Tween 80, which possesses a relatively high intrinsic viscosity. The presence of a greater amount of surfactant increases the viscosity of the continuous phase and enhances intermolecular interactions within the system. Elevated viscosity contributes to improved emulsion stability by restricting droplet mobility and delaying destabilization phenomena such as creaming and coalescence.

3.4. Colour

Visually, all nanoemulsion samples exhibited a yellow appearance, with color intensity varying among formulations (Figure 4). The lightness value (L*) increased significantly ($p < 0.05$) as the surfactant-to-oil ratio (SOR) increased from 1:1 to 3:1, which corresponds to a decreasing proportion of red palm oil in the formulation. This increase in L* indicates enhanced optical clarity at higher SOR levels.

The b* value, which represents yellowness, decreased with increasing SOR, reflecting the reduced contribution of red palm oil, the primary source of β-carotene, in the oil phase. Meanwhile, the hue angle (h*) ranged from 96.78 to 98.53, confirming that all nanoemulsions retained a yellow hue characteristic of β-carotene. Overall, these results demonstrate that the surfactant-to-oil ratio plays a significant role in determining the visual properties and optical clarity of β-carotene nanoemulsions.

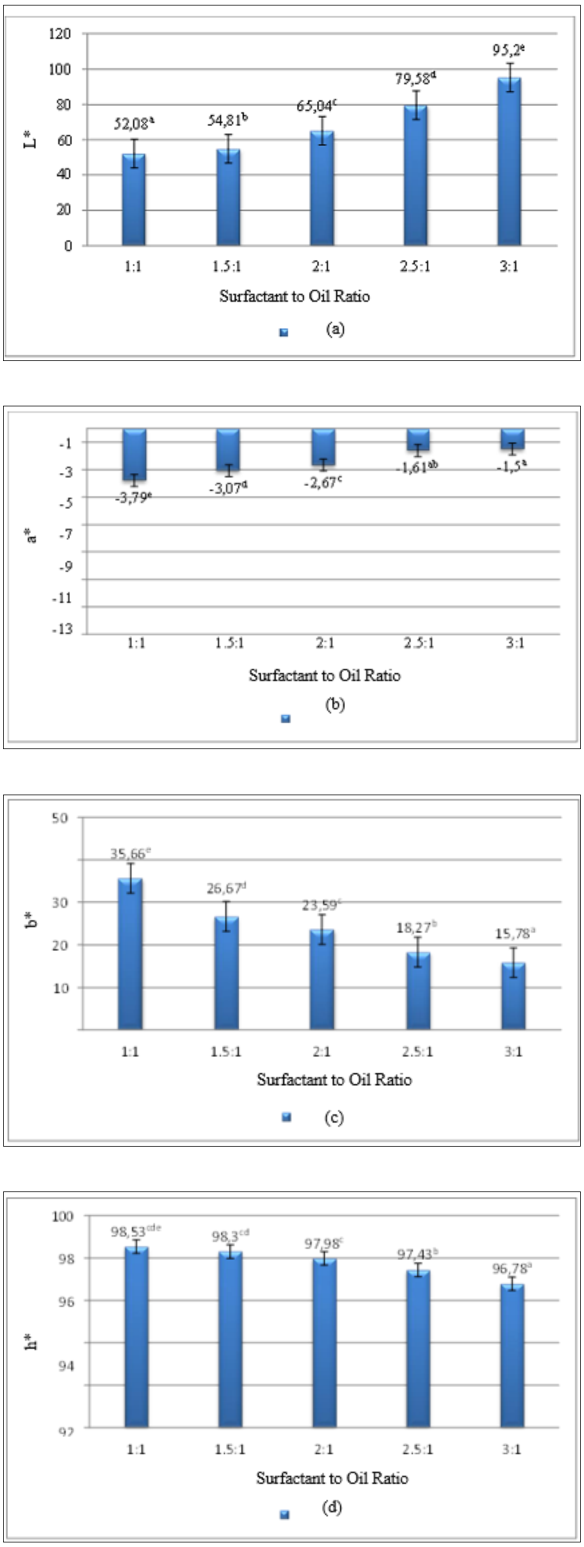

Figure 4. The effect of SOR on the parameters L* (a), a* (b), b* (c), and h* (d) in the sample

The hue angle (h*) values of the nanoemulsion samples differed significantly among formulations ($p < 0.05$). Hue angle values ranging from 96.78 to 98.53 confirmed that all nanoemulsions exhibited a yellow hue characteristic of β-carotene. The yellow coloration of the nanoemulsion samples can be attributed primarily to the presence of red palm oil as the oil phase, which is rich in β-carotene. As the nanoemulsions were formulated as oil-in-water (O/W) systems, the dispersed oil droplets containing β-carotene imparted a yellow appearance to the predominantly aqueous continuous phase.

3.5. β-carotene content

Based on the results presented in Figure 5, the β-carotene content of the nanoemulsions varied as a function of the surfactant-to-oil ratio (SOR). The highest initial β-carotene concentration was observed in the formulation with an SOR of 1:1, which contained the largest proportion of red palm oil (RPO) in the oil phase. As RPO is a well-established natural source of β-carotene, its higher proportion directly contributed to the increased initial pigment content in this formulation. Degradation of quality attributes during storage has been widely reported in various food systems, highlighting the importance of evaluating stability over time (Sulaiman & Khairi, 2024).

To better evaluate chemical stability, β-carotene retention during storage was expressed as a percentage of its initial concentration. All nanoemulsion formulations exhibited a gradual decrease in β-carotene retention, with a more pronounced decline observed after three weeks at room temperature. This reduction can be attributed to oxidative degradation and isomerization reactions induced by exposure to oxygen and ambient temperature, which disrupt the conjugated double-bond structure of β-carotene, leading to color fading and a loss of antioxidant activity. These findings are consistent with previous studies reporting the high susceptibility of β-carotene to oxidation under ambient conditions (Dewandari et al., 2019; Jo & Kwon, 2014; Qian et al., 2012).

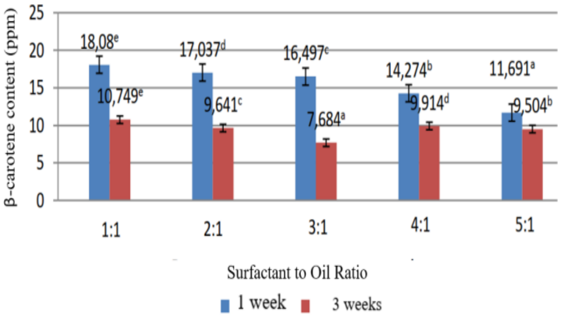

Figure 5. The effect of surfactan to oil ratio on the β-carotene content during the storage

The surfactant-to-oil ratio influenced the extent of β-carotene degradation and was closely associated with the physicochemical properties of the nanoemulsions. Formulations with higher SOR, particularly 3:1, demonstrated improved β-carotene stability compared to lower SOR systems. This behavior is consistent with the smaller droplet size and lower turbidity observed at higher SOR (Sections 3.1 and 3.2), which indicate the formation of a more homogeneous and stable nanoemulsion system. Smaller droplets provide a larger interfacial area that can be effectively covered by surfactant molecules, forming a compact interfacial layer that limits oxygen diffusion into the oil phase and reduces oxidative degradation. In addition, the relatively high magnitude of zeta potential (−32 to −42 mV) contributed to improved dispersion stability by preventing droplet aggregation. A more stable dispersion reduces the likelihood of droplet coalescence, which could otherwise accelerate β-carotene degradation due to localized oxygen exposure. This result probably explains why the nanoemulsion with SOR 3:1 retained higher β-carotene levels (11.69 ppm and 9.50 ppm after 1 and 3 weeks, respectively), despite having a lower initial β-carotene content than formulations with higher RPO proportions.

Despite this improvement, overall β-carotene retention remained relatively low across all formulations, indicating that it is inherently unstable under the applied storage conditions. This instability may also be influenced by prior heat exposure during red palm oil processing, as β-carotene is known to degrade at elevated temperatures (Jo & Kwon, 2014; Liu et al., 2014). Structural changes during processing and storage, including oxidation and isomerization, likely contributed to the nanoemulsions' pale-yellow appearance. Overall, these results demonstrate that increasing the surfactant-to-oil ratio improves both the physical and chemical stability of β-carotene nanoemulsions; however, further optimization is required to enhance β-carotene retention during storage.

4. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study demonstrated that a β-carotene nanoemulsion can be successfully formulated using a red palm oil–palm oil blend via a low-energy phase-inversion method. The modification of the oil phase by blending red palm oil with palm oil effectively reduced viscosity, enabling the formation of nanoemulsions with acceptable physicochemical characteristics. The surfactant-to-oil ratio was identified as a critical factor influencing nanoemulsion properties. Increasing the surfactant concentration led to smaller droplet size, lower turbidity, and improved dispersion stability, as reflected by stable zeta potential values. The optimal formulation was obtained at a surfactant-to-oil ratio of 3:1, which provided the best balance between physical stability and droplet uniformity. However, despite achieving good physical stability, the nanoemulsion system showed limited chemical stability, as evidenced by a significant decline in β-carotene retention during storage. This result suggests that while the developed system effectively stabilizes the nanoemulsion's physical structure, it is insufficient to protect β-carotene from oxidative degradation fully. Overall, this study highlights that the combination of oil phase modification and low-energy emulsification is a promising approach for developing nanoemulsion-based delivery systems. Nevertheless, further optimization is required to enhance the chemical stability of β-carotene for practical applications.

Recommendations

Future studies should focus on improving β-carotene stability through formulation and storage strategies, such as antioxidant incorporation, the use of co-surfactants, and controlled storage conditions (e.g., reduced temperature and light exposure). In addition, further investigation on bioavailability, antioxidant activity, and application in real food systems is necessary to evaluate the functional performance of the developed nanoemulsions.

ACKNOWLEDGMENTS

The authors thank to Universitas Mercu Buana Yogyakarta for financial support.

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