OPTIMIZING FORMULATION AND SYNTHESIS CONDITIONS OF RED PALM OIL (RPO)-BASED NANOEMULSIONS STABILIZED BY TWEEN 80

MEGAT TAUFIQ MEGAT NASIR [1], RICCA RAHMAN NASARUDDIN[1*], MARIATUL FADZILLAH MANSOR [1]

[1]*Department of Chemical Engineering and Sustainability, Kulliyyah of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur, Malaysia*

**Corresponding author: riccanasaruddin@iium.edu.my*

ABSTRACT: Nanoemulsion is an important class of nanomaterial that offers several advantages due to its improved stability and dispersibility in aqueous systems. In this preliminary study, oil-in-water nanoemulsions were synthesized using red palm oil (RPO). RPO was chosen for its high insoluble vitamin E content, a potent antioxidant that can reduce oxidative stress by neutralizing free radicals. The most suitable emulsifier, optimal formulation, and synthesis conditions to produce stable nanoemulsions were evaluated. A laser beam penetration test based on the Tyndall effect and light-scattering principles was used to confirm the presence of nanoemulsions qualitatively. The results indicate that Tween-80 produced the most stable and translucent nanoemulsion. The optimal formulation was found to have a weight ratio of 10:15:74:1 for RPO: Tween 80: water: glycerin. The optimal synthesis conditions were using the high-speed homogenizer at 15000 rpm for 40-minute synthesis time. The resulting nanoemulsions demonstrated stability suitable for further studies (e.g., physicochemical characterization, scale-up, and additional functionalization) for food and beverage applications.

KEY WORDS: *Oil-in-Water Nanoemulsions, Red Palm Oil, Tween 80, High-speed homogenizer, Formulation Optimization*

1. INTRODUCTION

Nanoemulsions are among the nanomaterials currently being developed for various industrial applications, particularly in food science, including nutraceuticals, packaging, food processing, safety, and nutrition [1,2]. Nanoemulsions are colloidal dispersions characterized by tiny droplet sizes, typically 20-200 nm [3,4,5,6], which contribute to their optical transparency and unique physicochemical properties. This nanoscale size not only imparts a transparent or translucent appearance but also enhances their stability, bioavailability, and functional performance in various applications [7]. Nanoemulsions are ideal for food and beverage applications due to their small droplet size, which enhances the bioavailability of encapsulated bioactive compounds. They also offer excellent physical stability against gravitational separation, creaming, and droplet aggregation [8,9]. Additionally, their high optical clarity makes them particularly appealing for use in transparent or mildly colored food and beverage systems [8,9]. In general, nanoemulsions are categorized into oil-in-water and water-in-oil types [4]. The oil-in-water nanoemulsion is preferably used in beverage formulations, as beverages are primarily water-based systems. Therefore, oil-in-water nanoemulsions are more compatible, more stable, and better suited for incorporating oil-soluble bioactive compounds into drinking products.

Fat-soluble vitamins such as A, D, E, and K play vital roles in human health but suffer from poor water solubility, low bioavailability, and susceptibility to degradation during processing and storage. Their incorporation into aqueous food and beverage systems remains a significant challenge. Currently, many vitamin drinks on the market contain vitamin A, C, B3, B6, B12, B5, potassium, zinc, chromium, and magnesium. [10]. Typically, vitamins A, D, E, and K are only needed in small amounts because they can be stored in cells, and excess intake can harm the consumer. Therefore, the application of oil-in-water nanoemulsions can be a promising delivery platform to enhance the solubility, stability, and bioavailability of these hydrophobic vitamins [11]. However, designing an effective, stable nanoemulsion as a carrier system for these fat-soluble vitamins requires formulation and process optimization.

Malaysia is among the world's largest producers of palm oil, making it a readily available and economically viable raw material for food and nutraceutical applications. However, the application of nanotechnology to palm oil remains limited, which constrains the utilization of this abundant resource for the development of advanced nanotechnology and nanomaterial-based applications. Therefore, this study focuses on the initial stage of developing and optimizing the formulation and synthesis conditions of oil-in-water nanoemulsions using red palm oil (RPO). RPO was selected as the main component of the oil phase because of its high content of natural antioxidants, particularly vitamin E. Vitamin E, in the form of tocopherols and tocotrienols, is also highly relevant to food and beverage applications due to its nutritional enhancement and oxidative stability properties [12]. No additional bioactive compound or other insoluble vitamins were added to the nanoemulsions in this study, as RPO was sufficient to demonstrate the concept of adding water-insoluble vitamins in nanoemulsions for potential food and beverage applications.

2. MATERIALS AND METHODOLOGY

2.1. Chemicals and Materials

Red palm oil (RPO) from Harvist Red Palm Oil company was purchased from a local grocer. Some emulsifiers, such as Tween 20, Tween 80, and soy lecithin, were purchased from EvaChem Sdn. Bhd. while Span 80 was purchased from Future Food Sdn. Bhd. Deionized water was used in all experiments and was obtained from the International Institute for Halal Research and Training (INHART Lab). The additive (glycerin) with a purity of 99.5% was purchased from Future Food Sdn. Bhd.

2.2. Synthesis and Characterization of Nanoemulsions

Nanoemulsions were prepared using several ingredients: oil, emulsifier, water, and glycerin. According to a study by Chong et al. (2018), the addition of a co-solvent such as glycerin (scientifically known as glycerol) can decrease the droplet size, thus producing more nanoemulsions. This effect is attributed to the co-solvent's ability to increase the viscosity of the aqueous phase and improve the solubility of the emulsifier [13]. All ingredients were weighed and transferred into 20 mL glass vials. The oil phase (RPO only) was slowly added to the water phase (water, emulsifier, and glycerin). Then, the final mixture was homogenized using a high-speed homogenizer at 15000 rpm for 30 min, with a synthesis time at standard room temperature (25 °C) and pressure (1 atm). The homogenization process was performed intermittently at 5-minute intervals to prevent the high-speed homogenizer rotor from overheating. All synthesized samples in this study were stored at standard room temperature (25 °C) and pressure (1 atm) for several days (up to 10 days) prior to characterization and stability evaluation. The appearance and presence of nanoemulsions in all synthesized emulsions were assessed on day 1 and day 10.

The formation of nanoemulsion was determined by the penetration of laser light through the solution based on the Tyndall effect and light scattering principles [14,15,16]. Based on the principles, when a laser beam can penetrate and be scattered through a solution, it is qualitatively confirmed that the system contains dispersed nanoparticles or nanodroplets [14,15,16]. This is a rapid, visual, and qualitative diagnostic tool, often used before confirming particle size using Dynamic Light Scattering (DLS) or Transmission Electron Microscopy (TEM). Meanwhile, Ultraviolet visible (UV-Vis) absorption measurement was done using JASCO 710 series UV-Vis spectrometer to compare two or multiple nanoemulsions. The intensity of the UV-Vis absorption is usually associated with the concentration of the nanoemulsions, while the wavelength of the UV-Vis absorption peak is usually associated with the dispersion size of the nanoemulsions [17,18].

2.2.1. Screening of Emulsifiers

Four emulsifiers (Tween 20, Tween 80, Span 80, and Soy lecithin) were screened. Each emulsifier was combined with water and glycerin, then RPO was added to the mixture. All emulsifiers were evaluated with a similar weight ratio of 5:5:89:1 for RPO, emulsifier, water, and glycerin, respectively. Each mixture with a varying emulsifier type was then homogenized using a high-speed homogenizer operated at 15000 rpm for 30 minutes, at standard room temperature (25 °C) and pressure (1 atm).

2.2.2. Optimization of Nanoemulsion Formulation

The best emulsifier identified in the screening process was Tween 80, which was subsequently used to optimize the nanoemulsion formulation. Several formulations, as shown in Table 1, were developed based on the weight percentage of all ingredients, ensuring that the combination of oil and emulsifier did not exceed 40 wt% [13]. The homogenizer mixing speed was kept constant at 15000 rpm, and the synthesis time was 30 minutes.

Table 1: Nanoemulsion formulations with different weight percentages of ingredients

No.	Red Palm Oil (wt. %)	Emulsifier (wt. %)	$R_{O:E}$ (wt. %)	Water (wt. %)	Glycerine (wt. %)	Process conditions
1	5	5	1:1	89	1	15000 rpm for 30 min
2	10	10	1:1	79	1	
3	20	20	1:1	59	1	
4	5	10	1:2	84	1	
5	10	15	2:3	74	1	
6	15	20	3:4	64	1	
7	10	5	2:1	84	1	
8	20	5	4:1	74	1	

2.2.3. Optimization of Synthesis Conditions

In this experiment, nanoemulsions were produced by the influence of different process conditions, but with the same formulation obtained from the previous experiment. The process conditions examined included mixing speed and synthesis time. Several mixing speeds, such as 10000, 15000, and 17000 rpm, were applied. Each speed was maintained for 30, 40, and 50 minutes of total synthesis time, as summarized in Table 2.

Table 2: Nanoemulsion synthesis with different conditions

Nanoemulsion Formulation	Mixing Speed (rpm)	Synthesis Time (min)
Selected formulation from Section 2.2.2.	10000, 15000, 17000	30
	10000, 15000, (17000, no result due to machine malfunctioned)	40
	10000, 15000, 17000 (No result due to machine malfunctioned)	50

3. RESULTS AND DISCUSSION

Nanoemulsions can be classified into two categories, which are oil-in-water and water-in-oil nanoemulsions, where the common liquids used to form this kind of emulsion are water and oil. For example, in the beverage industry, oil-in-water nanoemulsions are commonly used because of the aqueous nature of beverages. Nanoemulsions, characterized by their smaller droplet size, exhibit transparency and reduced light scattering in final products based on the Tyndall effect and light scattering principles. The translucent nature of nanoemulsions enables the incorporation of functional components without altering the visual appearance of the beverage. Results from this study include screening of emulsifiers and optimization of nanoemulsion formulation and synthesis conditions.

3.1. Screening of Emulsifiers

Fig. 1 shows four synthesized emulsions on day 1 and day 10. Based on the Tyndall effect and light scattering principles, when the laser beam can penetrate through a solution, it implies the presence of fine nanosized droplets [14,15,16]. In this study, emulsions prepared with Tween 20 and Tween 80 exhibited laser-beam penetration even at day 10, indicating the formation of stable, fine droplets in the nanoemulsions. RPO contains substantial amounts of reddish-orange carotenoid pigments, notably β-carotene. Its deep reddish orange color in bulk RPO shifted to brighter orange in nanoemulsions after being combined with hydrophilic emulsifiers such as Tween 20 and Tween 80 [13,19]. This color change was not primarily attributed to pigment degradation. However, the formation of nanoscale oil droplets significantly modifies light scattering and refractive-index relationship within the dispersion, thus altering the perceived hue and brightness of nanoemulsions compared to bulk RPO [12]. Furthermore, the addition of glycerin increases viscosity and adjusts the continuous phase's refractive index, which enhances light scattering and contributes to a perceptible orange shift in appearance [20,21].

In contrast, emulsions formulated with Span 80 and Soy lecithin remained conventional microemulsions, as evidenced by their turbid, milky appearance, and lacked the distinctive reddish-orange color of RPO. This turbidity indicates the formation of larger emulsions, which obscure carotenoid absorption, reduce optical transparency, and prevent laser beam penetration [12]. As a result, the carotenoids impart only a faint yellow tint, and the overall emulsion appears milky and yellowish white rather than an orange solution. On day 10, stronger laser light scattering, with no light penetration, also indicates the formation of larger emulsions when Span 80 and Soy lecithin were used as emulsifiers. The differences in droplet size are closely related to the hydrophilic-lipophilic balance (HLB) of each emulsifier. Tween 20 and Tween 80, with high HLB values (≈16.7 and 15.0, respectively), favor the formation of oil-in-water nanoemulsions, while Span 80 (HLB ≈ 4.3) and Soy lecithin (amphiphilic but more lipophilic) typically favor water-in-oil systems or coarse emulsions [22,23,24].

The observations on day 10 indicate that nanoemulsions prepared with Tween 80 maintained the highest transparency and laser beam penetration, confirming their presence. This observation aligns with a previous reported study by Chong et al. (2018), who optimized RPO nanoemulsions with a Tween 80/Span 80-stabilized system, achieving mean droplet sizes of approximately 120 nm [13]. Other recent studies also reported that using Tween 80 as an emulsifier produces smaller droplets and more stable nanoemulsions compared to lecithin-based emulsifiers [19,23]. Therefore, Tween 80 was selected as the primary emulsifier for subsequent optimization experiments in this study.

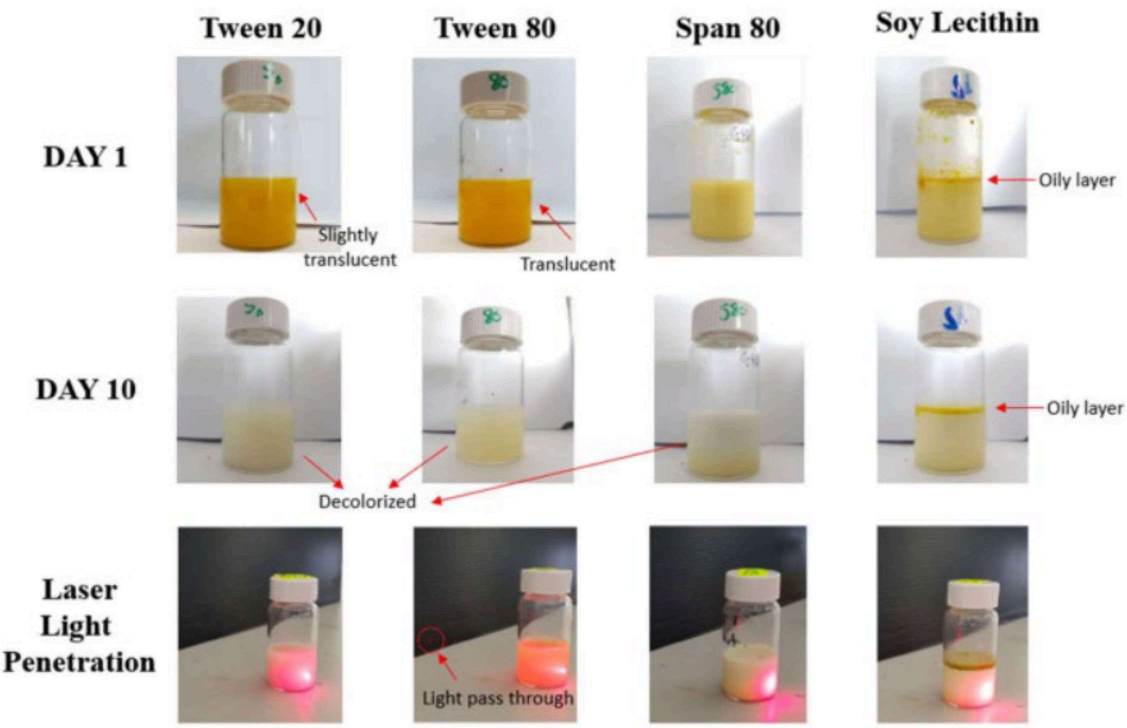

Fig. 1. Photos of emulsions synthesized using different emulsifiers at day 1 and day 10. The bottom photos show the presence of nanoemulsions when using Tween 80 as emulsifier based on laser light penetration through the solution at day 10.

Additionally, Fig. 1 indicates that the majority of nanoemulsions used in the emulsifier screening experiment were unstable on day 10, as evidenced by creaming and noticeable changes in solution color. Only nanoemulsions synthesized with Tween 80 appeared slightly translucent and light yellow. Despite the color change, laser beam penetration still occurred, probably because the nanoemulsions in the solution remained stable after 10 days. The stability of nanoemulsions refers to their ability to resist changes in system properties or environmental conditions over time. There are two main types of stability relevant to emulsions, which are thermodynamic stability and kinetic stability [25,26]. Thermodynamic stability means the system is in its lowest free energy state and thus does not spontaneously change or separate. Microemulsions are considered thermodynamically stable systems, characterized by spontaneous formation without external energy input and long-term stability under constant conditions [25,26].

In contrast, nanoemulsions are thermodynamically unstable. This means the free energy of the colloidal dispersion is higher than that of the separated phases, so they will eventually phase-separate if given enough time [27,28,29] as shown in Fig. 1. On the other hand, kinetic stability refers to the rate at which changes occur in the system. A kinetically stable system has a slow rate of destabilization processes such as creaming, flocculation, coalescence, or Ostwald ripening, enabling nanoemulsions to remain stable over practical time scales [28,29]. Nanoemulsions exhibit kinetic stability due to their small droplet sizes (typically 100–200 nm), which facilitates Brownian motion that counteracts gravitational separation and reduces droplet aggregation [28].

3.2. Optimization of Nanoemulsions Formulation

Next, the formulation of the ingredients was adjusted to achieve the best, most stable nanoemulsion. Eight formulations were evaluated as listed in Table 1. Fig. 2 (a and b) presents the appearances of synthesized emulsions for each formulation as well as the extent of laser light penetration through the samples. The formulations of nanoemulsions (Exp. 4, 5, and 6) in Fig. 2(a) maintained the same weight ratio 1:1 between RPO and emulsifier (Tween 80) but with increasing their combined weight percentage and decreasing water content. Nanoemulsions from Exp 1 with the lowest content of both RPO (5 wt.%) and Tween 80 (5 wt.%) and the highest water content (89 wt.%) exhibit the best appearance in terms of color and translucent characteristic. The laser light also penetrated these nanoemulsions on day 10. However, when the content of both RPO and Tween 80 increased to 20 wt. % (Exp. 2) and 40 wt. % (Exp. 3), the translucent appearance of synthesized emulsions was reduced, and the turbidity increased. Higher oil and emulsifier contents usually lead to larger droplet sizes or higher droplet volume fractions, resulting in greater light scattering. This reduces translucency and increases turbidity of the nanoemulsions [24].

Meanwhile, Fig. 2(b) shows the synthesized emulsion using formulations with different weight ratios between RPO and Tween 80. Based on Table 1, Exp. 4, 5, and 6 used more Tween 80 than RPO, with increasing total weight of these two ingredients. Meanwhile, Exp. 7 and 8 used lesser Tween 80 than RPO. All formulations in Exp. 4, 5, and 6 produced nanoemulsions with excellent translucency and bright orange color at day 1. However, laser light penetration tests revealed that only nanoemulsions from Exp. 4 and 5 allowed greater light transmission compared to nanoemulsions from Exp. 6. This observation is consistent with the results in Fig. 2(a), whereby increasing the content of both RPO and Tween 80 and decreasing the water content results in less formation of stable nanoemulsions.

On the other hand, when the Tween 80 content was reduced below the RPO content, as in Exp. 7 and 8, stable nanoemulsions could not be produced. An oil layer was observed on day 1 for these two formulations, likely due to an insufficient number of emulsifier molecules to reduce the surface tension of the oil components, leading to their separation from the water phase. The overall findings shown in Fig. 2(a and b) suggest that a higher Tween 80-to-RPO weight ratio contributed to a higher yield of stable nanoemulsions and a better translucent appearance. However, a higher combination of RPO and Tween 80 contents relative to water diminishes these positive effects. Accordingly, an optimal balance between oil, emulsifier and water is essential for producing high-quality nanoemulsions.

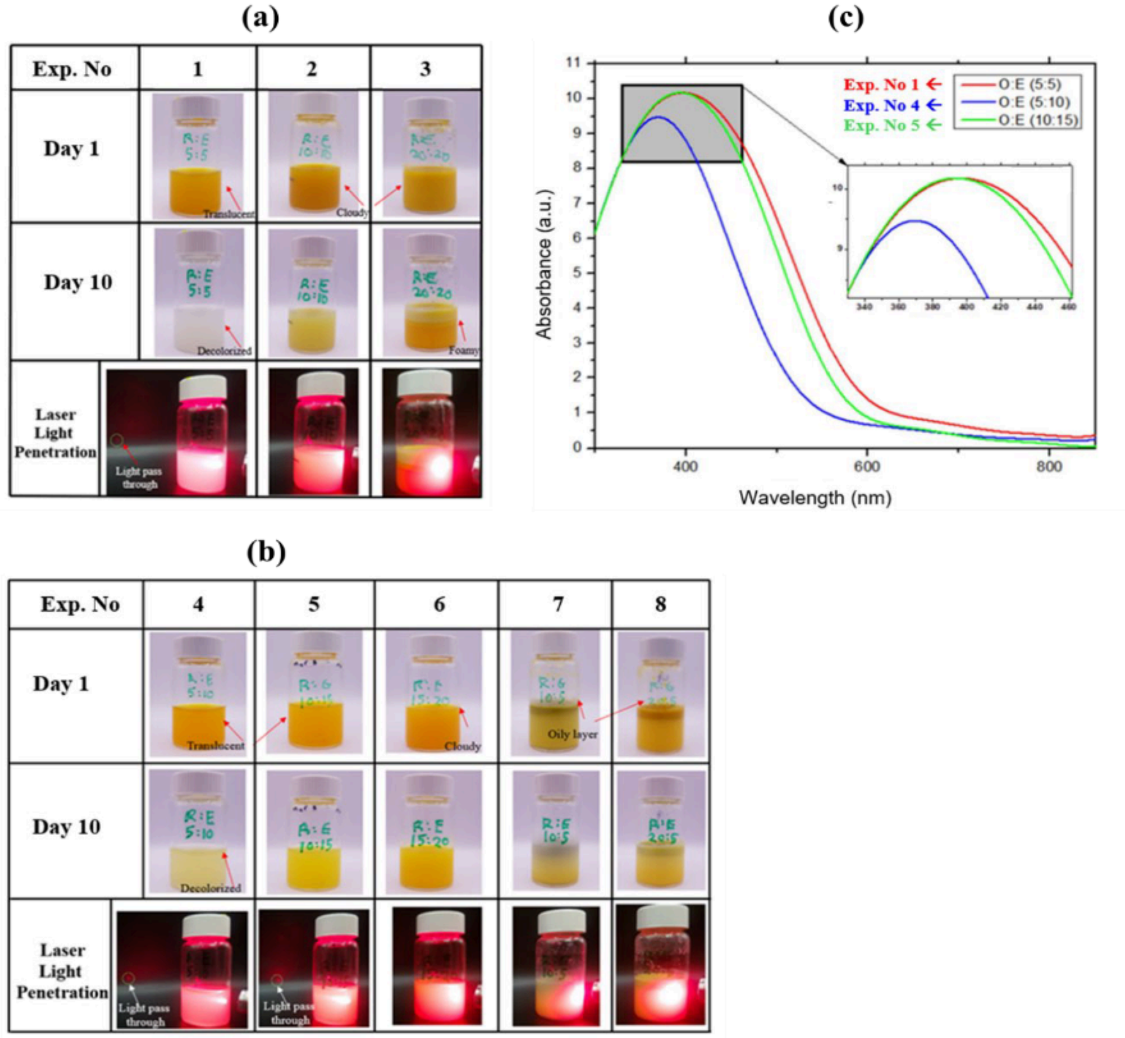

Fig. 2. (a) Appearance of nanoemulsion and penetration of laser light through the solution from Exp. 1, 2, and 3 with a similar weight ratio of RPO and Tween 80. (b) Appearance of nanoemulsion and penetration of light from Exp. 4, 5, and 6 with a higher weight ratio of Tween 80 than RPO and Exp. 7 and 8 with a lower Tween 80 weight ratio than RPO. (c) UV-Vis absorption spectra of selected nanoemulsions with different weight ratios of oils and emulsifier (O:E) from Exp. 1, 4, and 5. Refer to Table 1 for the details of each formulation. On day 10, only nanoemulsions from Exp 1, 4, and 5 exhibited clear laser beam penetration, and the UV-Vis absorption spectra differentiated the quality of the nanoemulsions.

After 10 days, nanoemulsions from Exp. 1, 4, and 5 were analyzed by UV–Vis absorption spectroscopy. Nanoemulsions with a similar and slightly higher content of Tween 80 than RPO (Exp. 1 and 5 with red and green graphs, respectively) showed stronger absorption (~10 a.u.) at ~410 nm. Meanwhile, those with Tween 80 at a two-fold higher weight ratio than RPO (Exp. 4, blue graph) had slightly lower intensity (~9 a.u.) at ~400 nm. According to the Beer-Lambert law, this suggests that too much Tween 80 compared to RPO could reduce the formation of nanoemulsions. The wavelength of the peak for Exp. 4 also shifted a bit to the lower wavelength, which could indirectly indicate a smaller size of nanoemulsions than those produced in Exp. 1 and 5. The excess Tween 80 in Exp. 4 likely provided better nanoscale stabilization of the oil droplets, preventing further growth and aggregation and thereby maintaining smaller droplet sizes. Actual size determination can be conducted in future studies to validate this explanation. Nevertheless, the 10:15 weight ratio between Tween 80 and RPO (Exp. 5) was chosen as the optimal formulation for further study. The main reason was the greater stability of the nanoemulsions, as evidenced by their better yellowish-orange color and greater laser light penetration, compared to those synthesized using other formulations. Sari et al. (2018) reported that their best formulation for producing RPO-based nanoemulsions stabilized by Tween 80 was using RPO at 5 wt. % only [19]. However, in this study, the best formulation had a higher RPO content (10 wt.%), resulting in higher Vitamin E levels, which is preferable for future food and beverage applications.

3.3. Synthesis conditions optimization

The optimal formulation from Exp. 5 produced nanoemulsions with RPO, Tween 80, water, and glycerin weight ratios of 10:15:74:1. This optimal formulation was used to optimize the mixing speed and the time taken to synthesize the nanoemulsions. Based on the results in Fig. 3, nanoemulsions prepared at 15000 rpm for 30 min, 17000 rpm for 40 min, and 15000 rpm for 40 min exhibited excellent translucency. On the other hand, nanoemulsions produced at a lower mixing speed of 10000 rpm for both 30 and 40 min turned cloudy on the first day and became turbid after 10 days, indicating that the nanoemulsion synthesis failed. Increasing the mixing speed from 15000 rpm to 17000 rpm slightly increased the nanoemulsion concentration, as shown by a 3.4% increase in UV-Vis absorbance intensity (from 9.86 to 10.21 a.u.). Maintaining a speed of 15000 rpm and extending the synthesis time from 30 to 40 min resulted in a 4.3% increase in UV-Vis absorbance intensity (from 9.86 to 10.28 a.u.). These results suggest that both factors enhance the concentration of nanoemulsions, with synthesis duration having a more substantial effect. The highest UV-Vis absorbance occurred at a longer wavelength (~420 nm), likely due to a slight increase in nanoemulsion size. Over a prolonged synthesis time (40 min), some smaller nanoemulsions might have aggregated in solution, leading to slightly larger nanoemulsion sizes.

Based on energy consumption consideration, 15000 rpm mixing speed and 40 min synthesis duration were chosen as the best conditions because they consumed slightly less energy than using 17000 rpm mixing speed for 30 min synthesis time. In addition to increased energy consumption, the higher mixing speed also increased the friction on the homogenizer's rotor, eventually leading to overheating and malfunction of the high-speed homogenizer. Thus, the synthesis conditions with a higher mixing speed for 40-minute synthesis time and all mixing speeds for 50-minute synthesis time could not be performed. Nevertheless, the current results still provide valuable insights into how mixing speed and synthesis duration affect nanoemulsion formation.

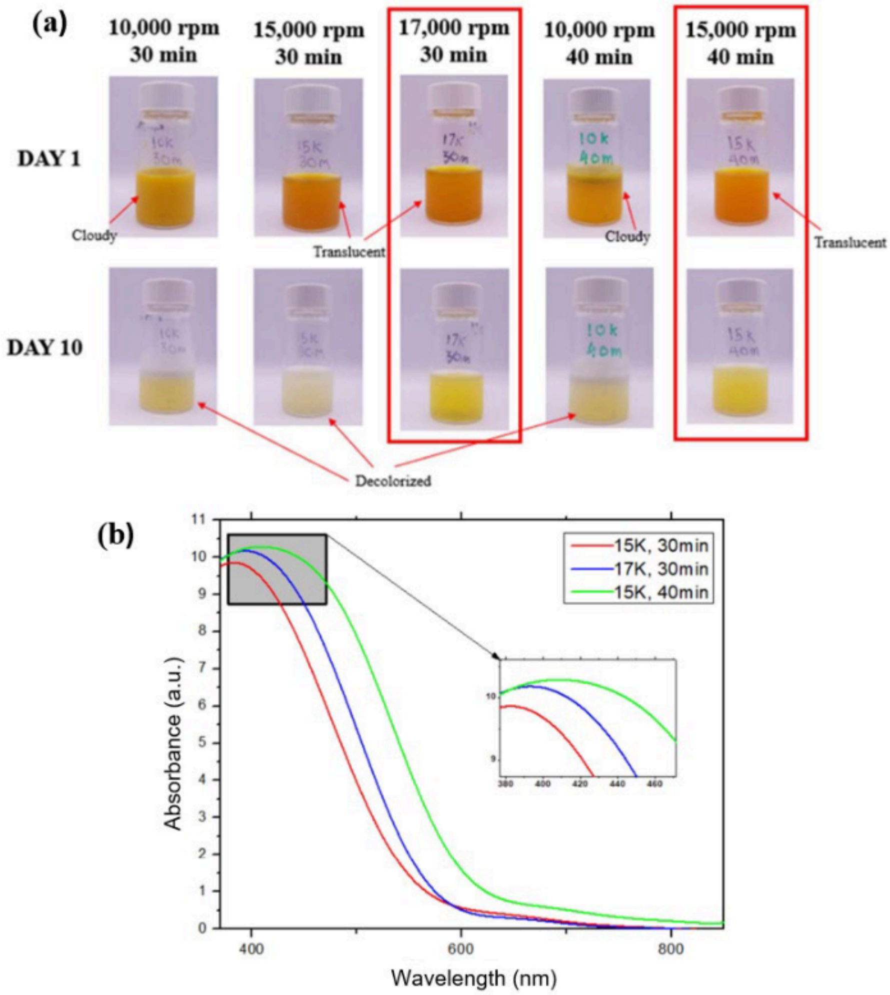

Fig. 3. (a) Appearance of nanoemulsions produced with different mixing speeds and synthesis times, and (b) UV-Vis absorption spectra of the nanoemulsions. Nanoemulsions in the red box had better appearances at day 1 and day 10, with the highest UV-Vis absorbance intensity in the green graph (longer duration), followed by the blue graph (higher mixing speed).

This study used a high-speed homogenization process, also known as rotor-stator emulsification (RSE), to produce nanoemulsions. This technique is widely applied to produce emulsions in various industries such as food, cosmetics, and pharmaceuticals. The rotor speed determines the hydrodynamic intensity and the resulting emulsion droplet size [29]. A notable reduction in mean droplet size is not typically observed after passing through the rotor-stator region, so multiple cycles are required to achieve a consistent droplet size, particularly for forming nanoemulsions with small droplets. Using only RSE can make it challenging to achieve high yield and stability in nanoemulsions compared with high-pressure homogenization, high-pressure microfluidic homogenization, or ultrasonic homogenization methods. However, RSE is generally less expensive than these alternative methods, making it more popular for industrial-scale production of emulsions and nanoemulsions [29].

4. CONCLUSION

In conclusion, different emulsifiers exert different effects on nanoemulsion formation. In this study, the incorporation of Tween 80 to produce nanoemulsions demonstrated outstanding results in terms of color, transparency, and penetration of laser light. The optimal formulation was found to have a weight ratio of 10:15:74:1 for RPO:Tween 80:water: glycerin. The optimal synthesis conditions were achieved under a mixing speed of 15000 rpm for 40-minute synthesis time. These optimal results produced the best nanoemulsions, as evidenced by their color, translucent appearance, stability, penetration of laser light, and the highest UV-Vis absorption intensity of 10.28 a.u. at the wavelength of ~420 nm. Overall, this work represents the optimization of formulation and synthesis conditions for nanoemulsions as a basis for future investigations into their physicochemical characterization and potential food and beverage applications.

ACKNOWLEDGEMENT

As supervisor, Dr. Ricca Rahman Nasaruddin acknowledges Megat Taufiq Megat Nasir for his contributions in sample preparation, experimental work, data collection, analysis, and the drafting of the initial article as part of his final-year project during his bachelor's degree. Ricca Rahman Nasaruddin also acknowledges Dr. Mariatul Fadzillah Mansor for her contribution in co-writing and reviewing the final manuscript.

REFERENCES

[1] Singh T, Shukla S, Kumar P, Wahla V, Bajpai VK, and Rather IA. (2017) Application of Nanotechnology in Food Science: Perception and Overview. Frontiers in Microbiology, 8
[2] Carpenter J, Pinjari DV, Kumar SV, Pandit AB. (2022) Critical Review on Hydrodynamic Cavitation as an Intensifying Homogenizing Technique for Oil-in-Water Emulsification: Theoretical Insight, Current Status, and Future Perspectives. Industrial & Engineering Chemistry Research, 61(30): 10587-10602.
[3] Shaikh NM VS, Nagoba SN, Shaikh AL. (2019) A Review on Current Trends of Nanoemulsion. Indo American Journal of Pharmaceutical Science, 06(01): 721-732.
[4] Malado G, Ande S, Chavhan S, Bartare S, Malode L, Manwar J, Bakal R. (2021) A Critical Reveiw on Nanoemulsion: Advantages, techniques and characterization. World Journal of Advanced Research and Reviews, 11(3): 462-473.
[5] Angelopoulou P, Giaouris E, Gardikis K. (2022) Applications and Prospects of Nanotechnology in Food and Cosmetics Preservation. Nanomaterials, 12(7): 1196.
[6] Delmas T, Piraux H, Couffin A-C, Texier I, Vinet F, Poulin P, Cates ME, Bibette J. (2011) How To Prepare and Stabilize Very Small Nanoemulsions. Langmuir, 27(5): 1683-1692.
[7] Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM. (2006) Nanoemulsions: formation, structure, and physical properties. Journal of Physics: Condensed Matter, 18(41): R635.
[8] Milinčić DD, Salević-Jelić AS, Kostić A, Stanojević SP, Nedović V, Pešić MB. (2024) Food nanoemulsions: how simulated gastrointestinal digestion models, nanoemulsion, and food matrix properties affect bioaccessibility of encapsulated bioactive compounds. Critical Reviews in Food Science and Nutrition, 64(22): 8091-8113.

[9] Ashaolu TJ. (2021) Nanoemulsions for health, food, and cosmetics: a review. Environmental Chemistry Letters, 19(4): 3381-3395.
[10] An U, Du X, Wang W. (2022) Consumer Expectation of Flavored Water Function, Sensory Quality, and Sugar Reduction, and the Impact of Demographic Variables and Woman Consumer Segment. Foods, 11(10): 1434.
[11] Crintea A, Dutu AG, Sovrea A, Constantin AM, Samasca G, Masalar AL, Ifju B, Linga E, Neamti L, Tranca RA, Fekete Z, Silaghi CN, Craciun AM. (2022) Nanocarriers for Drug Delivery: An Overview with Emphasis on Vitamin D and K Transportation. Nanomaterials (Basel), 12(8): 1376
[12] Aththar AF, Raushani F, Sekaringtyas FC, Muttaqien SE, Setyawati DR, Pratiwi RD, Rifa'i M, Rahmawati SI, Putra MY, Ahmadi P, Indriani DW, Ling JTS, Widyastuti E, Bayu A, Rosyidah A. (2025) Palm oil nanoemulsion enhances tocotrienol stability, antioxidant, and selective anti-melanoma activity. Naunyn Schmiedebergs Archives of Pharmacology, 2025 Jun 11.
[13] Chong W-T, Tan C-P, Cheah Y-K, B. Lajis AF, Habi Mat Dian NL, Kanagaratnam S, Lai O-M. (2018) Optimization of process parameters in preparation of tocotrienol-rich red palm oil-based nanoemulsion stabilized by Tween80-Span 80 using response surface methodology. PLOS ONE, 13(8): e0202771.
[14] Zaudin NAC, Sulaiman NS, Ting TC, Ramle SFM, Abdullah NH, Geng BJ. (2022) Development and characterization of nanoemulsion containing essential oil of Piper betle as the active ingredient via low energy emulsification method. AIP Conference Proceedings, 2454(1): 060051.
[15] Tseng K-H, Lin Y-H, Tien D-C, Ku H-C, Stobinski L. (2018) Stability analysis of platinum nanoparticles prepared by ESDM in deionised water. Micro & Nano Letters, 13(11): 1545-1549.
[16] Tseng K-H, Lin Z-Y, Chung M-Y, Tien D-C, Stobinski L. (2020) Parameter control and property analysis in the preparation of platinum iodide nanocolloids through the electrical spark discharge method. RSC Advances, 10(50): 30169-30175.
[17] Alafandi L, Nasaruddin RR, Engliman S, Mastuli MS. (2022) Green synthesis of silver nanoparticles using coffee extract for catalysis. Malaysian NANO-An International Journal, 1(2): 13-25.
[18] Amir D, Nasaruddin RR, Engliman NS, Sulaiman S, Mastuli MS. (2021) Effect of Stabilizers in the Synthesis of Silver Nanoparticles and Methylene Blue Oxidation. IOP Conference Series: Materials Science and Engineering, 1192(1): 012031.
[19] Sari F, Sinaga KR, Siahaan D. (2018) Formulation and Evaluation of Red Palm Olein Nanoemulsion. Asian Journal of Pharmaceutical and Clinical Research, 11(9): 237-240.
[20] Choi SJ, McClements DJ. (2020) Nanoemulsions as delivery systems for lipophilic nutraceuticals: strategies for improving their formulation, stability, functionality and bioavailability. Food Science and Biotechnology, 29(2): 149-168.
[21] Banasaz S, Morozova K, Ferrentino G, Scampicchio M. (2020) Encapsulation of Lipid-Soluble Bioactives by Nanoemulsions. Molecules, 25(17): 3966.
[22] Damayani IA, Paramita V, Yulianto ME. (2021) The Effect of HLB Surfactant Value on The Characteristics of Emulsion Biodiesel Palm Oil Using Homogenizer. Journal of Vocational Studies on Applied Research, 3(1): 9-13
[23] Kaur G, Singh P, Sharma S. (2021) Physical, morphological, and storage studies of cinnamon based nanoemulsions developed with Tween 80 and soy lecithin: A comparative study. Journal of Food Measurement and Characterization, 15(3): 2386-2398.
[24] Yalçinöz Ş, Erçelebi E. (2020) Effect of surfactant type and droplet size on lipid oxidation in oil-in-water nano-emulsions. Quality Assurance and Safety of Crops & Foods, 12(2): 1-11.
[25] McClements DJ. (2012) Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft Matter, 8(6): 1719-1729.

[26] Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, Aqil M, Talegaonkar S. (2009) Nanoemulsion Components Screening and Selection: a Technical Note. AAPS PharmSciTech, 10(1): 69-76.
[27] Kumar M, Bishnoi RS, Shukla AK, Jain CP. (2019) Techniques for Formulation of Nanoemulsion Drug Delivery System: A Review. Preventive Nutrition and Food Science, 24(3): 225-234.
[28] Souto EB, Cano A, Martins-Gomes C, Coutinho TE, Zielińska A, Silva AM. (2022) Microemulsions and Nanoemulsions in Skin Drug Delivery. Bioengineering, 9(4):158
[29] Liu Q, Huang H, Chen H, Lin J, Wang Q. (2019) Food-Grade Nanoemulsions: Preparation, Stability and Application in Encapsulation of Bioactive Compounds. Molecules, 24(23): 4242