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Characteristics of Microemulsion at Various Ratios of Surfactant–Polyethylene Glycol 400 and Red Palm Oil

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Abstract: *Red Palm Oil is a processed palm oil product rich in carotene (provitamin A) and vitamin E (tocopherols and tocotrienols), which are beneficial as antioxidants, anti-cancer agents, and cholesterol-lowering compounds. Red Palm Oil has potential as a raw material for microemulsion formulations in food and cosmetic applications. This study aimed to determine the effect of the surfactant–polyethylene glycol 400 ratio and red palm oil on microemulsion characteristics, as well as to identify the optimal ratio that produces the best microemulsion. This research is also expected to contribute to the field of agricultural product processing, particularly in the utilization of microemulsions combining surfactants, co-surfactants, and red palm oil, and to serve as a reference for the development of formulations for various applications, including cosmetics, skincare products, health supplements, and functional foods. The experiment employed a factorial Randomized Block Design (RBD) with two factors: surfactant: PEG 400 ratios (90:10, 70:30, 50:50) and surfactant–co-surfactant to red palm oil ratios (90:10, 87.5:12.5, 85:15), analyzed using ANOVA and Tukey's post hoc test at a 5% significance level. Results showed that the interaction of both factors had a highly significant effect on microemulsion characteristics, particularly on stability after 24-hour incubation and centrifugation stability. The best treatment was obtained at a surfactant:PEG 400 ratio of 90:10 and red palm oil ratio of 90:10, producing a clear microemulsion with a turbidity index of 0.216% after 24 hours, centrifugation stability of 0.199%, particle size of 13.4 ± 0.056 nm, and a polydispersity index (PDI) of 0.113. This microemulsion also showed stability across pH values (4.5–6.5) and dilutions (1:1, 1:9, 1:99), with transmittance values above 80% in most treatments. Hedonic sensory evaluation indicated an appearance score of 5.45 ± 0.605 (liked to very liked) and a low stickiness score of 1.35 ± 0.498 (disliked to strongly disliked), establishing it as the best treatment combination.*

Keywords: *Microemulsion, surfactant, co-surfactant, PEG 400, red palm oil*

I. Indroduction

Oil palm (Elaeis guineensis) is a strategic plantation crop with high productivity, serving as a major source of vegetable oil. Optimal production occurs after six years of age, yielding approximately 200 kg of fresh fruit bunches (FFB) per year, equivalent to 40 kg of crude palm oil (BPS Indonesia, 2022). As the world's leading palm oil producer, Indonesia had a plantation area of 15.93 million hectares in 2023, producing 47.08 million tons of FFB, an increase from 46.82 million tons in 2022 (BPS Indonesia, 2023). Crude palm oil (CPO) production reached 50.07 million tons, and palm kernel oil (PKO) 4.77 million tons [1], while exports of CPO and derivatives totaled 32.21 million tons or USD 30.32 billion, highlighting its economic significance (Ministry of Agriculture RI, 2024). [2].

Beyond crude oil, palm utilization extends to downstream industries such as cooking oil, margarine, oleochemicals, cosmetics, and bioenergy (Ministry of Industry, 2023). Red palm oil (RPO), produced without bleaching, retains carotene (provitamin A) and vitamin E (tocopherols and tocotrienols) as natural antioxidants [3]. These bioactive compounds offer health benefits, including antioxidant, anticancer, and cholesterol-lowering effects, though they are prone to degradation from oxidation, heat, and light exposure [3].

Microemulsion technology offers an effective approach to enhance the stability, solubility, and bioavailability of these bioactive compounds. Microemulsions are oil–water dispersions stabilized by surfactants and co-surfactants, forming micellar structures capable of encapsulating both lipophilic and hydrophilic molecules [4]. In this study, a combination of nonionic surfactants—Tween 80, Tween 20, and Span 80—at a 92:5.5:2.5 ratio, producing a total Hydrophilic–Lipophilic Balance (HLB) of 14.5, was adopted based on the optimal formulation reported by [5]. Polyethylene glycol 400 (PEG 400) was incorporated as a co-surfactant to reduce interfacial tension, enhance surfactant layer flexibility, and prevent droplet coalescence, thereby maintaining clarity and nano-sized droplet distribution [6].

Preliminary investigations in July 2024 indicated that surfactant–PEG 400 combinations produced clear and stable microemulsions at ratios of 90:10 to 60:40, whereas ratios of 50:50 to 10:90 tended to yield turbid systems. Consequently, this study examined surfactant–co-surfactant ratios combined with red palm oil at 90:10, 87.5:12.5, and 85:15 to evaluate their effects on physicochemical properties and stability and to determine the optimal formulation. This research provides a comprehensive understanding of PEG 400's role in stabilizing red palm oil microemulsions and establishes a foundation for developing formulations for cosmetics, skincare products, health supplements, and functional foods. These findings align with [7], who reported that similar microemulsion formulations of lemon peel essential oil remained clear and stable after 24-hour incubation, serving as a reference for further optimization and value-added applications of red palm oil.

II. Research Method

2.1 Place and Time of Research

This research was conducted at the Process Engineering and Quality Control Laboratory, the Food Analysis Laboratory, and the Food Processing Laboratory of the Faculty of Agricultural Technology, Udayana University, as well as at the PT. DKSH MESI Laboratory. The study was carried out from February 2025 to June 2025.

2.2 Experimental Design

In this study, the surfactant preparation procedure was adapted from Suhendra et al. (2012), who reported that a combination of Tween 80, Tween 20, and Span 80 in a ratio of 92:5.5:2.5 achieves an HLB value of 14.5 and optimal system stability. Each surfactant was weighed according to the specified ratio and mixed thoroughly to obtain a homogeneous surfactant solution with the desired HLB, which was then combined with red palm oil and co-surfactant, followed by the gradual addition of water under constant stirring to facilitate the formation of nano- to micrometer-sized droplets, ensuring optimal interfacial tension and system stability. In this study, the surfactant mixture was modified by adding PEG 400 as a co-surfactant in three different proportions (90:10, 70:30, and 50:50 v/v) to evaluate its effect on the formation, clarity, and stability of red palm oil microemulsions. Red palm oil was added at surfactant–co-surfactant ratios of 90:10, 87.5:12.5, and 85:15 in a volume of 5 mL, after which the mixture was stirred using a hot plate with a magnetic stirrer at 70°C ± 5°C, followed by the dropwise addition of 15 mL distilled water and incubation for 24 hours (Suhendra et al., 2012). After incubation, the microemulsions were evaluated for stability according to the observed parameters.

2.3 Variables Observed

The variables observed in this study included microemulsion stability after 24 hours of incubation, centrifugation stability, pH and dilution stability, particle size, and stickiness, which was evaluated using a hedonic test.

2.3.1 Microemulsion Stability After 24-Hour Incubation

The stability of the red palm oil microemulsion was evaluated after a 24-hour incubation period. Subsequently, turbidity index values and transmittance were measured using a UV/VIS spectrophotometer [8].

2.3.2 Centrifugation Stability

The centrifugation test was conducted by placing centrifuge tubes containing 10 mL of red palm oil microemulsion into a centrifuge and spinning them at 4000 rpm for 30 minutes. Following centrifugation, turbidity measurements were performed to assess stability [8].

2.3.3 pH and Dilution Stability

The stability of the microemulsion against pH and dilution was assessed by diluting the microemulsion with water and buffer solutions at pH levels of 4.5, 5.5, and 6.5 in ratios of 1:1, 1:9, and 1:99. The pH measurement was performed at room temperature using a pH meter. Prior to measurement, the electrode was calibrated using standard buffer solutions and then immersed into the sample. The pH value displayed on the screen was recorded. Subsequently, turbidity analysis was conducted to evaluate the stability of the diluted systems [8].

2.3.4 Storage Stability

The storage stability of the microemulsion was monitored at regular intervals every two weeks over a period of eight weeks. Stability was assessed through visual observation and turbidity index analysis [9].

2.3.5 Particle Size

Particle size analysis of the microemulsion was conducted using a Particle Size Analyzer (PSA). The sample was placed in a glass cuvette, and absorbance was measured to determine the globule diameter. The optimal microemulsion characteristics were indicated by the successful formation of a microemulsion at the highest red palm oil ratio [10].

2.3.6 Organoleptic Test

Organoleptic testing was conducted to assess the acceptability of red palm oil microemulsions, involving 20 semi-trained panelists and using the hedonic scale method. Panelists evaluated the samples based on sensory perceptions, assigning scores ranging from 1 to 6 to indicate their level of preference, which were then statistically analyzed to determine overall acceptance and sample characteristics. Specifically, the hedonic test assessed both texture and appearance of the microemulsions, where a score of 1 indicated "extremely disliked," 2 "disliked," 3 "slightly disliked," 4 "slightly liked," 5 "liked," and 6 "extremely liked" for each parameter. This method provided quantitative data on panelists' sensory preferences, enabling an objective evaluation of the quality and consumer acceptability of the microemulsion formulations [11].

III. Result and Discussion

3.1 Microemulsion Stability After 24-Hour Incubation

Analysis of variance indicated that the interaction among treatments had a highly significant effect ($P < 0.05$) on the stability of microemulsions after 24 hours of incubation, demonstrating that the combined ratios of surfactant–co-surfactant (Tween 80, Tween 20, Span 80 with PEG 400) and the proportion of red palm oil collectively influence the clarity and consistency of the microemulsion system. In other words, the effect on stability is not determined solely by the surfactant, co-surfactant, or oil individually, but by how these three factors interact; for instance, a specific surfactant–co-surfactant ratio may produce a stable microemulsion at one oil proportion but be less stable at another. The turbidity index (%) as an indicator of microemulsion stability is presented in Table 1, illustrating the degree of cloudiness or clarity for each formulation after incubation.

Table 1. Turbidity Index (%) of Microemulsion Stability After 24-Hour Incubation

Surfactant and PEG 400 Ratio	Red palm Oil (%)		
	(10)	(12,5)	(15)
90:10	0,216±0,013[a]	0,257±0,034[b]	0,389±0,003[c]
70:30	4,711±0,018[d]	4,751±0,046[d]	5,088±0,005[e]
50:50	5,333±0,011[f]	5,634±0,080[g]	5,966±0,024[g]

Analysis of variance revealed that the surfactant–co-surfactant ratio, red palm oil ratio, and their interaction had a highly significant effect on the turbidity index of microemulsions after 24 hours of incubation ($P < 0.01$), whereas the block factor was not significant ($P > 0.05$). The highest turbidity value was observed at a surfactant–co-surfactant ratio of 50:50 with red palm oil 85:15 (5.966 ± 0.024%), while the lowest values occurred at a 90:10 ratio with various oil proportions (0.216–0.389%), which, based on physical stability criteria, are classified as stable when <1% and visually clear [8]. Microemulsions with turbidity values >1% tend to experience droplet aggregation and cloudiness due to phase imbalance at high co-surfactant ratios, indicating that a higher surfactant proportion is required to maintain system stability and clarity. Previous studies have confirmed that clear microemulsion systems are characterized by a transmittance value of ≥90–95%, reflecting both the stability and clarity of the droplet dispersion [9].

3.2 Centrifugation Stability

Analysis of variance indicated that the interaction among treatments had a highly significant effect ($P < 0.05$) on the turbidity index after centrifugation, demonstrating that the combination of surfactant–co-surfactant ratios (Tween 80, Tween 20, Span 80 with PEG 400) and the proportion of red palm oil collectively influences microemulsion stability. In other words, the effect on stability is not determined solely by the surfactant, co-surfactant, or oil proportion individually, but by how these three factors interact; for instance, a specific surfactant–co-surfactant ratio may produce a stable microemulsion at one oil proportion but be less stable at another. The turbidity index values and the appearance of microemulsion stability after centrifugation are presented in Table 2.

Table 2. Turbidity Index (%) of Centrifugation Stability

Surfactant and PEG 400 Ratio	Red palm Oil (%)		
	(10)	(12,5)	(15)
90:10	0,199±0,005[a]	0,303±0,011[b]	0,357±0,055[b]
70:30	0,357±0,055[c]	3,580±0,119[c]	3,580±0,119[d]
50:50	4,081±0,130[e]	4,127±0,088[e]	6,023±0,148[e]

Analysis of variance revealed that the surfactant–co-surfactant ratio, the red palm oil ratio, and their interaction had a highly significant effect on the turbidity index of microemulsions after centrifugation ($P < 0.01$), whereas the block factor was not significant (P > 0.05). The highest turbidity value was observed at a 50:50 surfactant–co-surfactant ratio with red palm oil 90:10 (2.6155%), while the lowest values occurred at a 90:10 ratio with various oil proportions (0.0865–0.155%), indicating optimal stability at higher surfactant ratios. Formulations with lower surfactant ratios (70:30 and 50:50) exhibited increased turbidity, a cloudy appearance, and transmittance below 9%, reflecting instability due to droplet coalescence under centrifugal force. These findings are consistent with [8], who reported that stable microemulsion systems have turbidity <1% and a clear appearance. The principle of centrifugation supports droplet separation based on mass and size, where larger particles settle faster and smaller particles remain suspended [10], indicating that formulations with low surfactant content are more susceptible to reduced physical stability.

3.3 pH and Dilution Stability

The results of the analysis showed that the optimal red palm oil microemulsion treatments were not significantly affected (P > 0.05) by variations in pH and dilution ratios of 1:1, 1:9, and 1:99. The turbidity index (%) values and visual appearance of the red palm oil microemulsions under these conditions are presented in Table 3.

Table 3. Turbidity Index (%) and Visual Appearance of Red Palm Oil Microemulsions Under Various pH and Dilution Conditions

Dilution	Turbidity Index (%)			Transmittance		
	pH			pH		
	4,5	5,5	6,5	4,5	5,5	6,5
1:1	0,177	0,211	0,385	83,71	80,11	68,06
1:9	0,161	0,223	0,189	85	81,31	75,15
1:99	0,154	0,207	0,152	85,5	81,31	80,99

Analysis of variance indicated that variations in pH (4.5, 5.5, and 6.5) at each dilution level (1:1, 1:9, and 1:99) did not have a significant effect on the turbidity index of red palm oil microemulsions (P > 0.05), with all treatments belonging to the same group. Turbidity values ranged from 0.0655 to 0.0985%, remaining below 1%, and all samples appeared visually clear, indicating good stability against pH and dilution variations. This stability is supported by the use of nonionic surfactants, which are not affected by H^+ ion concentration, allowing the system to remain stable from acidic to neutral pH [16]. High transmittance values, particularly at pH 4.5 and 5.5 (80.11–85.50%), indicate homogeneous droplet dispersion, whereas the decrease at pH 6.5 (68.06%) may result from droplet enlargement without compromising visual stability. These findings are consistent with [17], who reported that high oil concentrations can increase turbidity and reduce clarity, yet properly formulated microemulsions maintain overall system stability.

3.4 Particle Size

The results of particle size stability testing for the red palm oil microemulsion, conducted using a Particle Size Analyzer (PSA), showed that the 90:10 ratio of surfactant–co-surfactant to red palm oil yielded a particle size of 13.4 ± 0.056 nm, with the most frequent droplet size measured at 12.3 nm. Dispersed systems with particle sizes below 100 nm are classified as microemulsions [16]. The particle size analysis results for the red palm oil microemulsion are presented in Table 4.

Table 4. Particle Size of Red Palm Oil Microemulsion

Peak No.	S.P Area Ratio	Mean	S.D.	Mode
1	100.0	13,4 nm	0,056nm	12,3 nm
Total	100.0	13,4 nm	0,056nm	12,3 nm

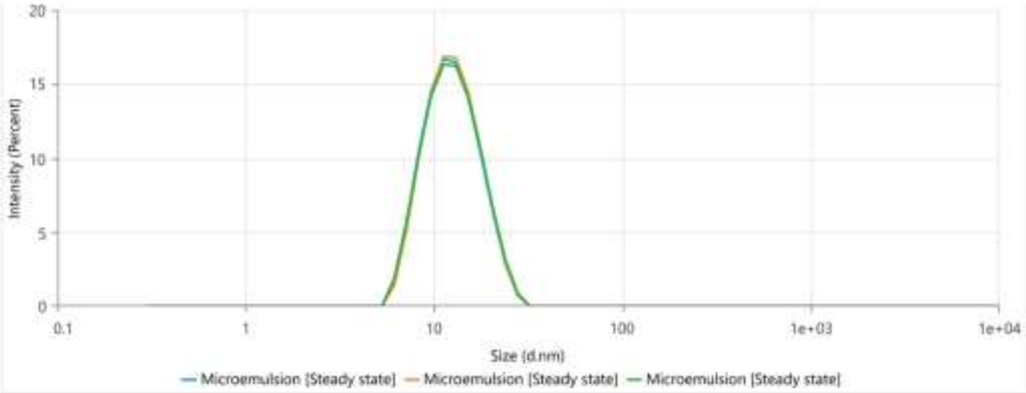

Figure 1. Particle Size Distribution of Red Palm Oil Microemulsion Formulated with a 90:10 Surfactant–Co-Surfactant Ratio

Figure 2 illustrates the particle size distribution graph of red palm oil microemulsion, with peak intensity observed in the 10–20 nm range. The three curves (blue, orange, and green) exhibit similar and symmetrical distribution patterns, indicating a monodisperse system and suggesting good physical stability. The particle size of <100 nm confirms that the system qualifies as a microemulsion rather than a coarse emulsion, which is typically associated with high turbidity and lower stability. The polydispersity index (PI) value of 0.113 further supports this conclusion, reflecting a uniform particle distribution. According to [17], systems with PI values ranging from 0.1 to 0.25 are considered monodisperse, and lower PI values correspond to enhanced system stability.

The extremely small particle size contributes to the clarity and transparency of the system, as the particles are too small to cause significant light scattering. In contrast, larger particles tend to increase light scattering, resulting in greater turbidity [18]. These findings reinforce that the red palm oil microemulsion formulation developed in this study produces a system with uniform particle size distribution in the nanometer range and high clarity, making it a promising candidate for active ingredient delivery applications in both food and cosmetic industries.

3.5 Storage Stability of Red Palm Oil Microemulsion at pH 4.5, pH 5.5, and pH 6.5 (Dilution 1:1)

The storage stability of red palm oil microemulsion was evaluated to assess the degradation rate over time through turbidity index (%) and transmittance measurements, conducted biweekly over an 8-week period. The microemulsion was tested under different pH conditions, namely pH 4.5, 5.5, and 6.5, using a 1:1 dilution ratio. Similarly, the microemulsion of lemon peel essential oil was evaluated under the same pH conditions. The stability test results demonstrated that the red palm oil microemulsion consistently exhibited a turbidity index below 1%, a clear appearance, and no visible sedimentation, indicating high physical stability throughout storage. The turbidity index values of the lemon peel essential oil microemulsion during storage under varying pH conditions are presented in Figure 2.

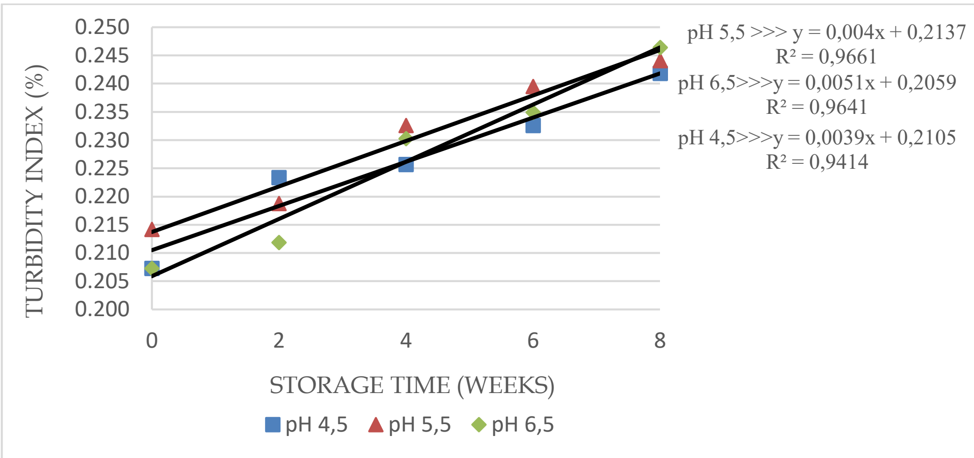

Figure 2. Stability of Red Palm Oil Microemulsion at Different pH Levels
Note: pH 4.5; 5.5; and 6.5 with 1:1 dilution over 8 weeks of storage (Turbidity Index Values)

Based on regression analysis, the stability of red palm oil microemulsions during 8 weeks of storage at a 1:9 dilution exhibited a linear increase in turbidity over time across all tested pH values (4.5, 5.5, and 6.5). The lowest rate of turbidity increase was observed at pH 4.5 (y = 0.0039x + 0.2105, R^2 = 94.14%), whereas pH 6.5 showed the highest rate (y = 0.0051x + 0.2059, R^2 = 96.41%), indicating the most significant decrease in stability. Although turbidity values exceeded 1%, the microemulsions remained visually clear, suggesting overall system stability. These findings support the theory that more acidic pH can slow the increase in turbidity by affecting droplet size, electrostatic interactions between particles, and the rate of oil oxidation [21]. Additionally, emulsions stabilized with nonionic surfactants maintain their electrical charge despite pH variations, enabling microemulsions to remain stable against dilution and centrifugation within the pH range of 4.5–6.5 [5].

3.5 Storage Stability of Red Palm Oil Microemulsion at pH 4.5, pH 5.5, and pH 6.5 (Dilution 1:1)

3.5.1 Appearance

Analysis of variance of the hedonic test indicated that the combination of surfactant–co-surfactant type and oil ratio had a significant effect on the acceptability of the appearance of red palm oil microemulsions ($P < 0.05$), including the interaction between these two factors. These findings suggest that formulation variations can significantly influence panelists' evaluations, whereas the block factor (panelists) was not significant ($P > 0.05$), indicating consistent assessments across panelists. Therefore, both the type of ingredient combination and their interaction independently contribute to determining the acceptability of microemulsion appearance.

Table 5. Organoleptic Evaluation Scores of the Appearance of Red Palm Oil Microemulsion

Surfactant and PEG 400 Ratio	Red Palm Oil (%)			Mean
	10	12,5	15	
90:10	5,45±0,605ᵃ	4,85±0,933ᵃ	4,00±0,795ᵃᵇ	3,67±1,728
70:30	3,55±0,887ᵃᵇᶜ	3,00±0,725ᵃᵇᶜ	1,60±0,681ᵇᶜᵈ	3,15±1,630
50:50	2,00±0,649ᵇᶜᵈ	1,60±0,681ᶜᵈ	1,10±0,308ᵈ	2,55±1,450
Mean	4,77±0,729	3,03±0,501	1,57±0,451	

Berdasarkan tabel 6, tingkat kesukaan kenampakan mikroemulsi minyak sawit merah tertinggi diperoleh pada rasio surfaktan–ko-surfaktan 90:10 dan rasio surfaktan–ko-surfaktan dengan minyak sawit merah 90:10 (5,45 ± 0,605; kategori agak suka–suka), sedangkan tingkat kesukaan terendah tercatat pada rasio 50:50 dengan minyak 85:15 (1,10 ± 0,308; kategori tidak suka–sangat tidak suka). Meskipun perbedaan antar perlakuan tidak signifikan pada taraf uji 5%, hasil ini menunjukkan bahwa komposisi surfaktan dan minyak berpengaruh terhadap homogenitas, kestabilan, dan kejernihan mikroemulsi. Formulasi dengan rasio 90:10 menghasilkan sistem emulsi lebih stabil dan homogen sehingga tampilan visual lebih menarik, sedangkan rasio 50:50 cenderung menimbulkan ketidakstabilan atau kekeruhan. Temuan ini sejalan dengan literatur yang menyatakan bahwa komposisi bahan sangat memengaruhi karakteristik fisik seperti tekstur dan kestabilan emulsi, sehingga pemilihan kombinasi bahan yang tepat penting untuk menghasilkan mikroemulsi yang optimal dan disukai konsumen [22].

3.5.2 Texture

Hedonic testing results indicated that the treatment of surfactant–co-surfactant and oil ratio had a highly significant effect ($P < 0.05$) on the texture acceptability of red palm oil microemulsions, whereas the panelist factor and the interaction between surfactant–co-surfactant and oil were not significant ($P > 0.05$), indicating relatively consistent evaluations among panelists. Therefore, these main factors independently influence panelists' preferences for microemulsion texture.

Table 5. Organoleptic Texture Evaluation Values of Red Palm Oil Microemulsion

Surfactant and PEG 400 Ratio	Red Palm Oil (%)			Mean
	10	12,5	15	
90:10	1,35±0,489ᵃ	1,85±0,366ᵃᵇ	2,45±0,945ᵃᵇ	3,08±1,776
70:30	3,00±1,026ᵃᵇᶜ	3,95±0,887ᵇᶜᵈ	4,45±1,099ᶜᵈᵉ	3,75±1,808
50:50	4,90±0,641ᵈᵉ	5,45±0,605ᵈᵉ	5,90±0,308ᵈᵉ	4,27±1,732
Mean	1,88±0,551	3,80±0,737	5,42±0,501	

Based on the study results, the highest acceptability for the texture of red palm oil microemulsions was observed in the combination of surfactant–co-surfactant ratio 50:50 and surfactant–co-surfactant to oil ratio 85:15 (5.90 ± 0.308, categorized as "like to very much like"), indicating a smooth, stable texture that aligns with sensory preferences. Conversely, the 90:10 combination for both ratios showed the lowest acceptability (1.35 ± 0.489, categorized as "dislike to very much dislike"). However, the formulations with the highest acceptability did not necessarily produce physically stable microemulsions;

thus, the selection of the optimal formulation was based on physical stability rather than solely on the highest preference scores. In general, increasing the surfactant–co-surfactant and oil concentrations tended to reduce texture acceptability, likely due to less homogeneous physical characteristics of the emulsion. The 50:50 and 85:15 combination produced a stable emulsion system with a smooth and uniform texture, whereas the 90:10 and 90:10 combination exhibited lower stability and less uniform texture, thereby reducing sensory acceptance. These findings are consistent with literature emphasizing that ingredient composition and formulation critically determine microemulsion texture quality and consumer acceptability [22].

IV. Interpretation of Analysis Result

The stability evaluation of red palm oil microemulsions revealed that the combination of surfactant–co-surfactant ratios (Tween 80, Tween 20, Span 80 with PEG 400) and oil concentration significantly influenced the physical properties, including turbidity, clarity, and consistency. After 24-hour incubation, the 90:10 surfactant–co-surfactant ratio with 10–15% red palm oil exhibited the lowest turbidity index (0.216–0.389%) [Table 1], remaining below the stability threshold of 1% and visually clear. In contrast, formulations with 50:50 surfactant–co-surfactant ratios, particularly at higher oil content (15%), showed the highest turbidity (5.966%), indicating droplet aggregation and phase imbalance due to excessive co-surfactant content. These results confirm that the stability of microemulsions is determined by the interaction between surfactant, co-surfactant, and oil proportions rather than individual components.

Centrifugation stability tests reinforced these findings. Formulations with a high surfactant ratio (90:10) maintained turbidity below 1% across all oil concentrations (0.0865–0.357%), while lower surfactant ratios (70:30 and 50:50) exhibited increased turbidity (up to 6.023%) and cloudy appearance [Table 2], reflecting instability due to droplet coalescence under centrifugal stress. This observation aligns with prior reports that stable microemulsions should remain clear (turbidity <1%) and resist particle aggregation under applied stress [8].

The red palm oil microemulsions also demonstrated robust stability against pH variations (4.5–6.5) and dilutions (1:1, 1:9, 1:99). Turbidity values remained below 1% with high transmittance (68.06–85.50%) [Table 3], indicating homogeneous droplet distribution and clear appearance across tested conditions. The lowest turbidity increase was observed at pH 4.5, while pH 6.5 showed slightly higher turbidity, likely due to minor droplet enlargement without compromising visual stability. These results are attributed to the nonionic nature of the surfactants, which maintain system stability across acidic to neutral pH ranges[5].

Particle size analysis of the optimal formulation (90:10 surfactant–co-surfactant with 10% oil) revealed a mean droplet size of 13.4 ± 0.056 nm and a mode of 12.3 nm, with a polydispersity index of 0.113 [Table 4, Figure 1]. The monodisperse distribution and sub-100 nm droplet size confirm the classification as a microemulsion and contribute to high transparency, minimizing light scattering and enhancing stability.

Storage stability over eight weeks at 1:1 dilution showed a linear increase in turbidity over time across all tested pH values. The slowest increase occurred at pH 4.5 ($y = 0.0039x + 0.2105$, $R^2 = 94.14\%$), whereas pH 6.5 displayed the highest rate ($y = 0.0051x + 0.2059$, $R^2 = 96.41\%$), indicating that slightly acidic conditions better preserve stability. Despite turbidity exceeding 1% in some cases, all formulations remained visually clear, indicating overall system stability during storage [Figure 2].

Sensory evaluation complemented the instrumental data. The highest appearance acceptability was recorded for the 90:10 surfactant–co-surfactant with 10% oil (5.45 ± 0.605, "like–slightly like"), while the lowest was observed in the 50:50 with 85:15 oil formulation (1.10 ± 0.308, "dislike–very much dislike") [Table 5]. Texture evaluation showed the 50:50, 85:15 combination achieved the highest scores (5.90 ± 0.308, "like–very much like"), despite lower physical stability, whereas 90:10 and 90:10 formulations exhibited the lowest texture scores (1.35 ± 0.489), reflecting a trade-off between sensory preference and physical stability [Table 6]. These findings emphasize that optimal formulation selection should consider both physical stability and sensory acceptance, as higher surfactant ratios favor system clarity and stability, while higher oil and co-surfactant ratios may enhance texture but reduce physical stability [22].

V. Conclusion

The results of this study demonstrated that the stability of red palm oil microemulsions was significantly influenced by the interaction between surfactant–PEG 400 ratios and oil concentration during 24-hour incubation and centrifugation, whereas variations in pH and dilution did not exert a significant effect on the turbidity index. Sensory evaluation revealed that a 50:50 surfactant–co-surfactant ratio combined with an 85:15 surfactant–co-surfactant to oil ratio produced microemulsions with the smoothest, most homogeneous, and stable texture, although panelist preference did not always correspond with physical stability. Notably, the formulation with a 90:10 surfactant–co-surfactant ratio and 90:10 oil proportion exhibited optimal physical and organoleptic characteristics, including clear appearance, low turbidity (0.216% after 24-hour incubation), high centrifugation stability (0.199%), and a mean particle size of 13.4 nm. This system also demonstrated resilience to pH and dilution variations, particularly at pH 5.5 with a 1:9 dilution, achieving transmittance values exceeding 80% and maintaining high clarity. Hedonic assessment further confirmed favorable appearance (5.45 ± 0.605, "like to very much like") and low stickiness (1.35 ± 0.489, "dislike to very much dislike"), indicating that this formulation provides both excellent microemulsion stability and visual acceptability. These findings underscore the critical role of optimizing surfactant, co-surfactant, and oil ratios to achieve microemulsions with superior physical properties and sensory quality, providing a foundation for the development of red palm oil microemulsions in cosmetic, skincare, functional food, and health supplement applications.

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