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Eco-Friendly Nanoemulsions of Orange Peel Essential Oil: Surfactant Optimization and Long-Term Colloidal Stability

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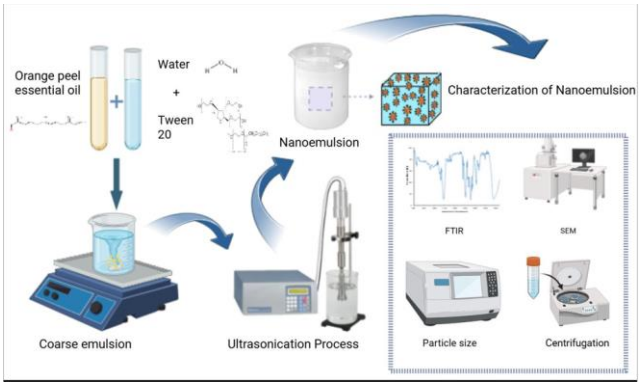
Graphical abstract



Abstract

Orange (*Citrus sinensis L.*) peel essential oil (OPEO) exhibits strong antioxidant and antimicrobial properties, supporting its potential use in diverse food and pharmaceutical applications. However, its industrial use is limited due to its hydrophobic nature and poor aqueous dispersion. This study developed a nanoemulsion system to overcome this limitation using ultrasonic emulsification with OPEO and sesame oil (SO), which was stabilized by the addition of Tween 20 surfactant. Various oil-to-surfactant ratios (1:1, 1:2, 1:3, and 1:4 v/v) and oil concentrations were evaluated for their effects on droplet size and kinetic stability. The optimized formulation at a 1:4 (v/v) and 80% OPEO in the oil phase showed the smallest droplet size (83.66 nm), low polydispersity index (PDI) (0.24), and a highly negative zeta potential (-31.93 mV), indicating enhanced physical stability. Kinetic stability was confirmed by centrifugation and long-term storage, with no phase separation observed for up to 60 days. SEM images revealed well-dispersed spherical droplets, and FTIR analysis confirmed the successful interaction and encapsulation of oil components. The nanoemulsion retained pH (7.22 to 6.08) and antioxidant activity (58.80 to 57.50%) during storage. This study addresses the formulation challenges of essential oil nanoemulsions and offers a stable delivery system for potential food and edible coating applications.

Keywords: Nanoemulsion, colloidal stability, orange peel essential oil, oil-to-surfactant ratios, droplet size, sesame oil, ultrasonic emulsification

1. Introduction

Essential oils are aromatic blends of bioactive components derived from aromatic plants. These secondary metabolites possess strong antimicrobial and antioxidant activity and serve as functional ingredients (flavor enhancers) in many food applications (Guerra-Rosas *et al.* 2016). The production and utilization of essential oils, a by-product of the Citrus family, are increasing day by day.

OPEO is a widely utilized essential oil in the pharmaceutical, food, and cosmetic industries. The OPEO composition typically consists of limonene (94%), linalool (0.5%), neral (0.1%), octanal (0.4%), myrcene (2%), geraniol (0.1%), decanal (0.4%), and other constituents (Hashtjin & Abbasi 2015). Furthermore, these primary constituents of OPEO include alcohols (linalool), terpenoids (limonene), esters, and aldehydes (octanal, decanal), exhibit less molecular weights (Jing *et al.* 2014) and enhance the volatility of essential oils.

Due to their distinctive aromas and cost-effectiveness, the utilization of such compounds has consistently been considered by the food industry. However, their practical application is limited by poor solubility, incompatibility with food matrices, and instability under processing and storage conditions. Therefore, innovative strategies are needed to enhance their stability and functionality in food systems. This represents a pivotal advancement in manufacturing, trade, and utilization of aromatic flavors and compounds in both food and non-foodstuffs (Ikarini *et al.* 2025).

In this context, emulsion technology, particularly the nanoemulsion method, emerges as a vital process for improving the nanoencapsulation, solubility, and protection of aromatic compounds (Silva *et al.* 2012). These suspensions contain tiny oil drops coated with an emulsifier, dispersed in the water phase. These kinetically stable nanoemulsions have <200 nm average droplet size (Liu *et al.* 2022). The nanosized droplets showed

distinctive characteristics for their use in the food industry (Do *et al.* 2020). Primarily, nanoemulsions exhibit a transparent appearance, which allows the lipophilic bioactive compounds incorporated into optically clear products, including various foods and beverages (Yalçınöz & Erçelebi 2018). Nanoemulsions gained notable attention due to their beneficial features, including high solubility, biodegradability, physical stability, bioavailability, and environmental friendliness (Nasr *et al.* 2022). Novel purification and formulation methods can tailor nanoemulsion systems for targeted food and pharmaceutical applications, ensuring better encapsulation efficiency and long-term performance (Pi *et al.* 2025).

Tween 20, a food-grade non-ionic surfactant approved for food and pharmaceutical applications, was selected due to its high emulsification efficiency, safety profile, and compatibility with essential oil-based nanoemulsions. .Tween 20, was selected due to its rapid adsorption onto oil droplet surfaces and its strong ability to reduce interfacial tension, thereby minimizing droplet coalescence and enhancing nanoemulsion stability (Degner *et al.* 2014). Tween 20 has a higher hydrophilic-lipophilic balance (HLB = 16.7) compared to Tween 80 (HLB = 15.0), making it more suitable for forming oil-in-water nanoemulsions, particularly when encapsulating hydrophobic compounds like essential oils. Its lauric acid tail provides faster interfacial adsorption and can lead to the formation of smaller, more uniform droplets. Additionally, Tween 20, is recognized for its low toxicity and is widely utilized in pharmaceutical and food formulations(Vinaya *et al.* 2021).

Nanoemulsions, being thermodynamically unstable systems, are prone to phase separation, primarily through the phenomenon known as "Ostwald ripening" (McClements & Rao 2011). Therefore, different ripening inhibitors have been incorporated in nanoemulsion formulations to prevent the system from creaming, Ostwald ripening, flocculation, and coalescence. Sesame (*Sesamum indicum*) is the earliest crop harvested in sub-tropical and tropical areas globally, and sesamolin and sesamin are identified as its primary components. SO was selected as the carrier oil due to its excellent oxidative stability, biocompatibility, and natural antioxidant content, which complements the properties of OPEO and enhances the overall stability and functionality of the nanoemulsion system (Lammari *et al.* 2020). Numerous studies have highlighted the synergistic impacts of SO with different oils; it acts as a ripening inhibitor, which inhibits the Ostwald ripening process and extends the shelf life of nanoemulsion (Prakash *et al.* 2019). However, in this study, the synergy between SO and OPEO has been created to address the volatility and stability challenges of nanoemulsion formulations.

In formulating the nanoemulsion system, particular consideration was given to process efficiency, scalability, and the use of sustainably sourced ingredients. Compared to conventional methods such as high-pressure homogenization or rotor-stator mixing, ultrasonic emulsification is a relatively cost-effective and energy-efficient approach, making it suitable for scalable nanoemulsion production. It facilitates the formation of smaller droplets and enhances kinetic stability without requiring high surfactant concentrations, making it ideal for thermally sensitive compounds such as essential oils (Zhou *et al.* 2022). Furthermore, the use of OPEO derived from citrus processing byproducts contributes to the valorization of the agri-food waste streams. This dual focus on energy-efficient processing and waste-derived functional ingredients enhances the overall sustainability of the system, aligning with current trends in environmentally responsible food formulation. However, this project has been designed in urgent demand to create approaches for the incorporation of OPEO into food systems while improving its stability and effectiveness. By characterizing the physicochemical properties, antioxidant activity, and morphological features of nanoemulsions made from OPEO and SO, this study aims to bridge the gap between the functional benefits of essential oils and their practical application in the food sector. Ultimately, this study will contribute towards the adoption of flavoring agents and functional ingredients that encourage healthier food choices and endorse sustainable practices in food production.

2. Materials and methods

2.1. Procurement of raw material

OPEO was extracted by the hydrodistillation method in the Rosemary extraction lab, Horticulture Department, University of Agriculture, Faisalabad, Pakistan. Cold-pressed SO was purchased from local manufacturer Khalispur (Lahore, Pakistan). Tween 20, DPPH reagent (2,2-diphenyl-1-picrylhydrazyl), ethanol, and ultrapure water were purchased from Sigma Aldrich (St. Louis, MO, USA). Ultrapure water was used for the preparation of all solutions.

2.2. Extraction of OPEO

Hydrodistillation is used for the extraction of essential oil from orange peels. In an alembic system, peels were dipped in distilled water, bringing the mixture to a boil under atmospheric pressure, and volatile aromatic compounds were released from plant cells. This azeotropic mixture (combination of water and volatile aromatic compound) is evaporated at constant pressure, then condensed and separated in a Florentine flask because of immiscibility and density difference. A cohobating system is used for recycling of distilled water in a siphon to improve the quality and quantity of essential oil (Golmohammadi *et al.* 2018).

2.3. Preparation of Nanoemulsion

Nanoemulsion was formulated using OPEO and SO as the oil phase, with the non-ionic surfactant Tween 20 and water. The SO amount was fixed at 1% v/v relative to the total emulsion volume in all nanoemulsion treatments. The coarse emulsion was formulated by mixing surfactant and oil in different ratios (v/v) in water as mentioned in **Table 1** by using a magnetic stirrer at 700 rpm for 10

minutes. Afterward, coarse particles were further decreased to nano size by subjecting the coarse emulsion to ultrasonic emulsification 20 kHz Sonicator (Ultrasonics, USA), 750 W power output. The sonicator probe was dipped in coarse emulsion and sonicated the mixture for 180 seconds. The temperature change should be less than 10°C between the initial coarse emulsion and the final nanoemulsion. The heat produced throughout the ultrasonication procedure was lessened by placing the sample bottle in an ice bath. Consequently, all nanoemulsion treatments were characterized by performing different analyses to assess the stability of the nanoemulsion. All characterization processes were conducted at room temperature.

Table 1. Volume-based composition (v/v %) of nanoemulsions prepared using different oil-to-surfactant ratios. The oil phase consisted of OPEO and SO, and the aqueous phase included distilled water

Treatment	OPEO (%)	SO (%)	Tween20 (%)	Water (%)
NE_1	2	1	3	94
NE_2	4	1	5	90
NE_3	2	1	6	91
NE_4	4	1	10	85
NE_5	2	1	9	88
NE_6	4	1	15	80
NE_7	2	1	12	85
NE_8	4	1	20	75

NE: Nanoemulsion OPEO: Orange peel essential oil SO: Sesame oil.

2.4. Characterization of nanoemulsion

2.4.1. Determination of particle size and polydispersity index (PDI)

Particle size and PDI value were evaluated with the help of a dynamic light scattering instrument with Zetasizer software. Samples were diluted in distilled water to prevent the multiple scattering phenomenon before analysis (Prakash *et al.* 2019).

2.4.2. Determination of Zeta-potential

Zeta-potential was determined by using a dynamic light scattering instrument with Zetasizer software. It is generally used for examining droplet charge and samples were diluted in distilled water to prevent the multiple scattering phenomenon before the analysis (Prakash *et al.* 2019).

2.4.3. Stability of nanoemulsion

Nanoemulsion stability was initially assessed through centrifugation at 10,000 rpm for 30 minutes, followed by storage at 25 °C to monitor treatments for any phase separation, as described by (Ghosh *et al.* 2013).

2.4.4. Scanning electron microscopy (SEM)

SEM analysis was performed to collect the structural information of nanoemulsion by following the method of Periasamy *et al.* (2016). SEM was conducted at the Central Hi-Technology Laboratory of the Government College University, Faisalabad, Pakistan.

2.4.5. Fourier transform infrared spectroscopy (FTIR)

Alteration in the chemical bonding of oil molecules in nanoemulsion was assessed through FTIR analysis. The FTIR spectra of nanoemulsions under different conditions were examined in the range from 4000 to 600 cm^{-1} by FTIR spectrophotometer (Bruker, Tensor 27, America). Before the analysis of each sample, optimization of the equipment was done by an empty scan, and for each sample, the FTIR spectra were generated based on an average of 10 scans.

2.4.6. Determination of pH

The pH of the nanoemulsions was determined using a pH meter (LMPH-12, Labman) at 25°C.

2.4.7. Antioxidant activity of nanoemulsion

The antioxidant activity was assessed by the DPPH method, as described by Farshi *et al.* (2017) with slight modification. In this procedure, a 1 mL nanoemulsion sample was mixed in 2 mL of ethanolic (0.1 mM) DPPH solution. After incubation for 60 min, centrifuge the solution at 10,000 rpm for 15 min and check the absorbance at 517 nm through a UV-visible spectrophotometer. The antioxidant activity was calculated using the following equation (Equation 1):

$$\text{Antioxidant activty} = 1 - \frac{\text{Sample absorbance}}{\text{control absorbance}} \times 100 \quad (1)$$

2.5. Storage stability of nanoemulsion

Nanoemulsion treatments were transferred in amber-colored glass bottles, tightly sealed, and stored at 25°C for 60 days. Then, their stability was assessed by performing the above-mentioned analysis after 20 days interval. At specific storage intervals, all treatments were subjected to centrifugation test, only stable treatments of nanoemulsion were selected for further analysis.

2.6. Statistical analysis

All analyses were performed in triplicates and outcomes were presented as mean and standard deviation. ANOVA followed by Tukey multiple comparison post hoc test and GraphPad Prism (version 10.2.3) was used to determine the statistically significant differences among all group means ($p < 0.05$).

3. Results and discussion

3.1. Particle size

Particle size was a critical parameter for evaluating the nanoemulsion stability. The particle size had a great impact on the functional and physicochemical attributes of nanoemulsions such as chemical reactivity, morphology, and stability (Perumal *et al.* 2021). The particle size of nanoemulsion treatments with different oil-to-surfactant ratios is presented in **Table 2**. The results revealed that surfactant concentration and particle size have an inverse relationship. The prepared nanoemulsion with different oil-to-surfactant ratios at 1:1 (v/v) showed the highest particle size NE_1 (152.04 nm) and NE_2 (144.48 nm). However, the results have been found according to the following trend 1:4<1:3<1:2< 1:1 (v/v), as the oil to surfactant ratio was enhanced, the particle size was reduced. By increasing the oil-to-surfactant ratio, the

particle size of the nanoemulsion was significantly reduced, indicating enhanced emulsification efficiency. Similar trends were reported by Smejkal *et al.* (2021) and Carpenter and Saharan (2017), where optimized oil-to-surfactant ratios resulted in smaller droplet sizes. In the current study, stable nanoemulsions NE_7 and NE_8 were successfully formulated at a 1:4 (v/v) oil-to-surfactant ratio, resulting in the least particle size of 94.45 nm and 83.66 nm, respectively. The selection of oil and surfactant concentration has a significant impact on the final results of prepared OPEO nanoemulsions. The small droplet size (83.66 nm) achieved in the optimized nanoemulsion significantly enhances the surface area, which can improve the bioavailability of the encapsulated OPEO by facilitating better absorption in biological systems. Moreover, nano-sized droplets enhance physical stability and optical transparency, making the formulation more suitable for incorporation into food systems without altering texture or appearance.

Table 2. Physicochemical characterization of nanoemulsion (Mean ± S.D)

Treatments	Particle size (nm)	PDI	Zeta potential (mV)	pH	Antioxidant activity (%)
NE_1	152.04±1.93^a	0.74±0.02^a	-5.15±0.90^a	7.21±0.03^b	54.97±0.14^c
NE_2	144.48±2.01^b	0.70±0.01^b	-6.48±0.87^a	7.05±0.03^c	58.66±0.07^a
NE_3	135.77±3.04^c	0.60±0.03^c	-11.64±1.41^b	7.16±0.02^b	58.70±0.10^a
NE_4	125.30±1.97^d	0.53±0.05^d	-15.78±1.05^c	7.10±0.02^c	55.53±0.06^b
NE_5	114.14±1.52^e	0.47±0.03^e	-17.45±1.11^c	7.19±0.04^b	55.72±0.06^b
NE_6	108.20±1.54^f	0.38±0.02^f	-22.85±2.39^d	7.15±0.01^b	55.60±0.13^b
NE_7	94.45±2.72^g	0.28±0.02^g	-28.84±1.37^e	7.13±0.04^{bc}	55.74±0.07^b
NE_8	83.66±2.10^h	0.24±0.03^h	-31.93±1.66^f	7.22±0.03^a	58.80±0.07^a

NE: Nanoemulsion, PDI: Polydispersity Index, Values represent mean ± standard deviation (n = 3). Superscript letters within each column indicate statistically significant differences (p < 0.05) using one-way ANOVA followed by Tukey's HSD test.

The optimized concentration of OPEO is very important for obtaining stable nanoemulsions with the lowest particle size, as shown in the results all formulations with 80% OPEO have smaller particle sizes as compared to other formulations containing 66% OPEO. When the OPEO concentration increased from 80% the low-quality nanoemulsions were produced i.e., less stable and non-transparent due to the Ostwald ripening phenomenon (Nirmal *et al.* 2018). Similar findings were reported by (Do *et al.* 2020).

Although NE_8 initially showed the smallest particle size (83.66 nm), it exhibited a moderate size increase to 105.81 nm after 60 days. The size growth can be attributed to the onset of Ostwald ripening over extended storage. While no phase separation occurred, the gradual increase in droplet size indicates partial destabilization. SO was incorporated at a fixed concentration (1% v/v) acted as a ripening inhibitor by mitigating this effect. Mechanistically, SO is rich in long-chain triglycerides that are virtually insoluble in water, reducing the chemical potential difference between small and large droplets. Additionally, non-polar lipid chains in SO exhibit strong molecular compatibility with the hydrophobic components of OPEO, such as limonene and other terpenoids. This structural compatibility leads to a uniform oil phase, which slows down the diffusion-driven mitigation of oil molecules between droplets, thereby inhibiting Ostwald ripening. While SO effectively delayed destabilization, its fixed low concentration might not have been sufficient to completely halt droplet growth over extended storage. These findings align with prior studies, which emphasize the importance of both the composition and concentration of oil-phase components in determining nanoemulsion stability (Chang & McClements 2014; Chuesiang *et al.* 2018)

During storage at ambient temperature, the particle size of all nanoemulsion samples was increased as shown in **Figure 1**. No phase separation was observed till 20 days of the storage period, after that phase separation occurred in some treatments. The increased particle size was associated with the lower quantity of surfactant present in the emulsifying chamber. With the limited quantity of surfactant, smaller particles aggregate due to the process of coalescence (Mehmood *et al.* 2017) and lastly, larger particles have been generated. Similar findings were observed by Rao and McClements (2011), during storage of early 4 weeks, the particle size of nanoemulsion enhanced due to coalescence (particle aggregation) and Ostwald ripening phenomena. However, the treatments NE_7 (152.20) and NE_8 (105.81) prepared with 1:4 (v/v) oil-to-surfactant ratio remained stable among all treatments of nanoemulsion after 60 days storage period at room temperature. A similar trend has been found by Sh *et al.* (2015) as the tween 20 concentration increased, the transparency and stability of nanoemulsion samples increased.

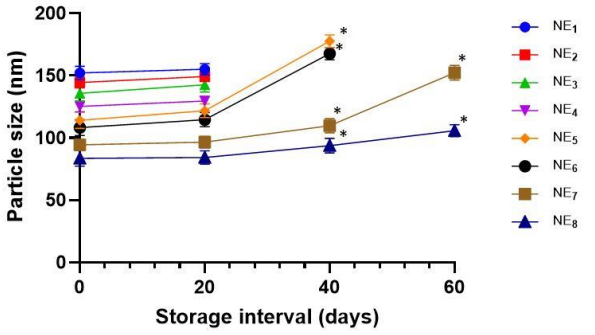


Figure 1. Particle size (nm) of nanoemulsion during 60 days of storage interval at 25°C (Mean ± S.D)

3.2. Polydispersity index (PDI)

The homogeneous particle size was directly associated with the PDI value of nanoemulsion. The PDI value closer to 1 and 0 indicates the heterogenous and homogenous behavior of particle distribution in the nanoemulsion system (Kang & Song 2018; Topuz *et al.* 2016). The PDI value of nanoemulsion treatments with different oil-to-surface ratios is presented in **Table 2**. The prepared nanoemulsion with different oil-to-surfactant ratios at 1:1 (v/v) showed the highest PDI values NE_1 (0.74) and NE_2 (0.70). However, the results have been found according to the following trend 1:4<1:3<1:2<1:1 (v/v), as the oil-to-surfactant ratio enhanced the PDI value was reduced and homogeneity of particle size distribution increased. The nanoemulsion NE_7 and NE_8 prepared with 1:4 (v/v) obtained lower PDI values 0.28 and 0.24 respectively. However, the particles were disrupted, re-arranged and re-coalesced due to the ultrasonic wave progression in emulsion. The breakage of interaction effect among the particles and coalescence disturbs the kinetic stability and particle size distribution of nanoemulsion. Moreover, factors such as the composition of essential oil, nanoemulsion development method, and oil-to-surfactant ratios are the main factors that influence the mean particle size and its distribution (Modarres-Gheisari *et al.* 2019). Rinaldi *et al.* (2017) reported a less than 0.3 PDI value of neem oil nanoemulsion prepared with Tween 20. Perumal *et al.* (2021) reported that green tea essential oil nanoemulsion developed with optimized ultrasonic parameters had 0.273 PDI value.

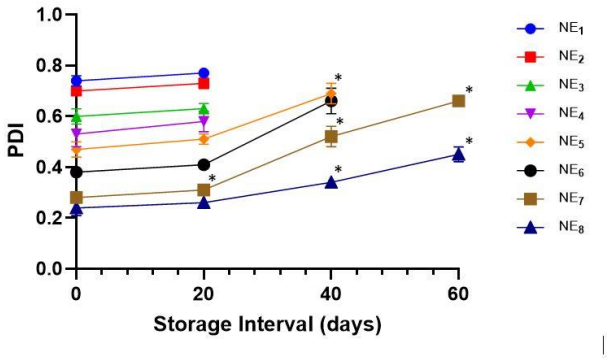


Figure 2. PDI of nanoemulsion during 60 days of storage interval at 25°C (Mean ± S.D)

During storage at ambient temperature, the PDI value of all nanoemulsion samples was increased due to the non-homogeneous behavior of particle size distribution in nanoemulsion as shown in **Figure 2**. The large particle sizes have a larger surface area for the interaction of particulates and show high potential for shattering surfactant film on the contacted surface of droplets, and as a result, coalescence occurred (Nazarzadeh *et al.* 2013). The PDI value of all treatments was significantly increased after 20 days storage period, except the 1:4 (v/v) oil-to-surfactant ratios treatments. Slight changes in PDI value have been observed in NE_7 (0.28 to 0.63) and NE_8 (0.24 to 0.45) from 0 to 60 days of storage period. During the storage PDI value of cinnamon oil nanoemulsion showed a rising trend by following an almost constant pattern (Yuliani *et al.* 2018). The findings revealed that nanoemulsions move towards lower kinetic stability due to weak interaction in the nanoemulsion system during the storage period. These results correlated with the present study because the coalescence rate was enhanced during the storage period, subsequently, the PDI value increased, and the system faced kinetic instability.

3.3. Zeta Potential

The zeta potential is an important factor for checking the nanoemulsion stability. It measures the electrical charge (repulsion/attraction) among particles and the main reason for dispersion, flocculation or agglomeration in nanoemulsion systems (Dickinson 2009). However, the stable range of zeta potential for particles is > ±30 mV (Arredondo-Ochoa *et al.* 2017). The zeta potential of nanoemulsion treatments with different oil-to-surface ratios is presented in **Table 2**. The prepared nanoemulsion with different oil-to-surfactant ratios at 1:1 (v/v) showed lower zeta potential NE_1 (-3.72 mV) and NE_2 (-6.48 mV). The results followed the trend 1:4>1:3>1:2>1:1 (v/v), indicating a direct relationship between the oil-to-surfactant ratio and the zeta potential of the nanoemulsion particles. The nanoemulsion NE_7 and NE_8 prepared with 1:4 (v/v) obtained the highest zeta potentials -28.84 mV and -31.93 mV, respectively. The nanoemulsions possess negative charge due to the presence of non-ionic surfactant (adsorption of negative ions on oil surface) and the existence of functional groups in the composition of OPEO and SO. The particles of basil oil nanoemulsion were stable due to the presence of non-ionic surfactant even though the absolute magnitude of particle charge was low (Tang *et al.* 2012). Gorjian *et al.* (2022) also reported that the spearmint essential oil nanoemulsion had negative zeta potential -34.2 mV, due to the presence of non-ionic surfactant tween 20.

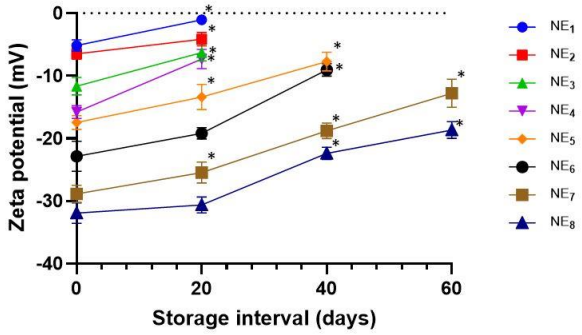


Figure 3. Zeta potential (mV) of nanoemulsion during 60 days of storage interval at 25°C (Mean ± S.D)

During storage at ambient temperature, the zeta potential of all nanoemulsion samples was decreased due to the reduction of electrostatic repulsion and particles aggregated in nanoemulsion as shown in **Figure 3**. The relative decrease in the absolute values of zeta potential over time might be ascribed to the repulsion in dispersion and particles were agglomerated or flocculated (Islam *et al.* 2023). The zeta potential of all treatments was significantly decreased after 20 days of storage period except the 1:4 (v/v) oil-to-surfactant ratio treatments. However, slight changes in zeta potential have been

observed in NE_7 (-28.84 to -12.78 mV) and NE_8 (-31.93 to -18.64 mV) from 0 to 60 days of storage period. Similar findings had been observed by Sadeghian *et al.* (2023) and Gorjian *et al.* (2022), during storage a relative reduction in zeta-potential was examined in all treatments of nanoemulsion due to lowering the electrostatic charge among particulates and starting agglomeration.

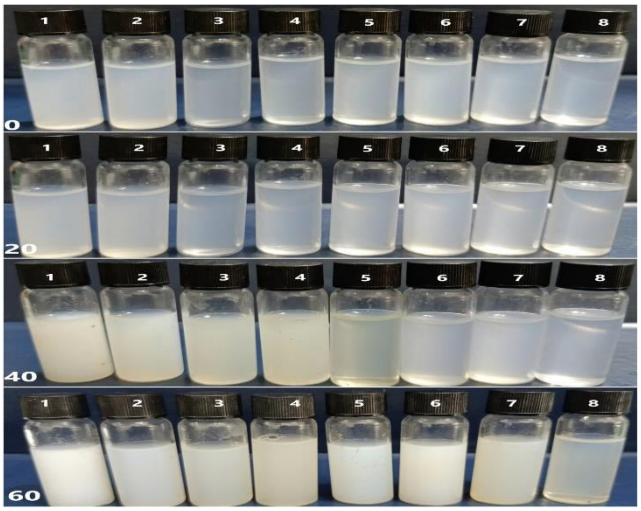


Figure 4. Appearance of nanoemulsion during 60 days of storage interval at 25°C

3.4. Centrifugation

Surfactant provides steric hindrance which sustains the repulsion among particles and the processing method is useful for decreasing particle size and controlling polydispersity of suspension particles. The synergistic effect of surfactant and ultrasonication helps protect the nanoemulsion stability under the shear forces of centrifugation. Centrifugation was conducted to assess the stability of the nanoemulsion. All treatments of nanoemulsions remained optically transparent and no phase separation occurred after centrifugation at 10,000 rpm at 0 day as shown in **Figure 4**. Asadinezhad *et al.* (2019) demonstrated that no phase separation showed in orange oil nanoemulsion after centrifuging at 9000 rpm for 30 mins. Carpenter and Saharan (2017) described the ultrasonication impact on nanoemulsion and they reported that formulations developed with optimized ultrasonic conditions had no phase separation after centrifugation.

During storage at ambient temperature, the separation trend of all nanoemulsion samples was described in **Table 3.** All treatments were visibly stable after centrifugation at 20 day intervals, after that some treatments faced phase separation because the nanoemulsion system is thermodynamically unstable which indicates that colloidal dispersion has more free energy as compared to water and oil phases individually, therefore particle size enhanced and nanoemulsion disrupted over time. But the 1:4 (v/v) oil to surfactant ratio treatments (NE_7 and NE_8) remained physically stable after the centrifugation test till 60 days of storage period as shown in **Figure 4.** Ghosh *et al.* (2013) also elaborated that nanoemulsion treatments were stable during one month of storage period and particle size also remained constant without any phase separation in nanoemulsion samples. However, at specific storage intervals, only stable treatments of nanoemulsion were selected for further analysis.

Table 3. Nanoemulsions pass the centrifugation test during 60 days of storage interval at 25°C

Treatments	Storage interval (days)			
	0	20	40	60
NE_1	Stable	Stable	Unstable	Unstable
NE_2	Stable	Stable	Unstable	Unstable
NE_3	Stable	Stable	Unstable	Unstable
NE_4	Stable	Stable	Unstable	Unstable
NE_5	Stable	Stable	Stable	Unstable
NE_6	Stable	Stable	Stable	Unstable
NE_7	Stable	Stable	Stable	Stable
NE_8	Stable	Stable	Stable	Stable

NE: Nanoemulsion

3.5. SEM

High resolution pictures of nanoemulsion were obtained with the help of SEM analysis. The SEM images of stable treatments of nanoemulsions NE_7 and NE_8 are displayed in **Figure 5**. Pictures showed that particles of nanoemulsions were very small, uniformly distributed distinct spherical shapes and had no huge cluster in suspension. This inspection reveals that optimized oil and surfactant amounts selected in this study were effective in developing highly uniform and homogeneous nanoemulsions.

3.6. FTIR

The structural characteristics of nanoemulsion treatments were assessed through FTIR spectroscopy, with spectra of NE7 and NE8 compared against pure OPEO and SO to evaluate successful encapsulation are presented in **Figure 6**. Key vibrational bands in the nanoemulsions, such as the broad O-H stretch near ~3380 cm^{-1}, C-H stretching around 2917-2928 cm^{-1}, and C=C stretching at 1631 cm^{-1}, were evident in all formulations. However, compared to pure OPEO and SO, the nanoemulsion treatments displayed slight peak shifts and intensity modulations, particularly in the fingerprint region (1250-1000 cm^{-1}), indicating intermolecular interactions and encapsulation effects.

Characteristic peaks corresponding to C-H stretching (2917 cm^{-1}), C=C stretching (1631 cm^{-1}), CH2 bending (1460 cm^{-1}) and C-O stretching (1086 cm^{-1}) were present in both NE_7 and NE_8 formulation exhibited a stronger and sharper C-O peak at (1086 cm^{-1}) attributed to effective entrapment of oxygenated compounds (e.g., limonene, sesamolin) within the emulsified droplets. The

attenuation and merging of some peaks, especially in the NE8 spectrum, further suggest encapsulation of bioactive components from OPEO and SO into a unified nanoemulsion matrix.

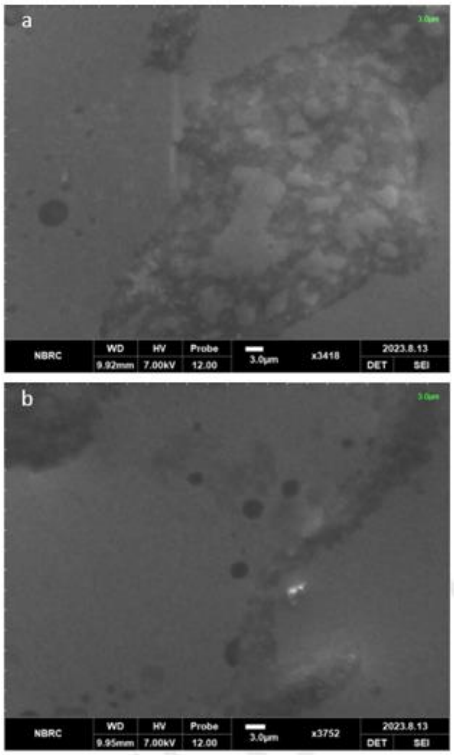


Figure 5. Scanning electron micrograph of stable treatments of nanoemulsion 1:4 (v/v) oil-to-surfactant ratios (a) NE_7 (b) NE_8 Scale bar = 3.0 µm.

The spectral differences between nanoemulsions and their respective pure oil components validate the physical entrapment of OPEO and SO, supported by changes in functional group vibrations. These modifications can be linked to successful nanoencapsulation, where surfactant interactions protect sensitive compounds from environmental degradation during storage. These findings align with previous studies demonstrating that Tween-based nanoemulsions after spectral properties due to molecular-level stabilization of hydrophobic compounds (Cantika *et al.* 2023; Ortiz-Tafoya & Tecante 2018).

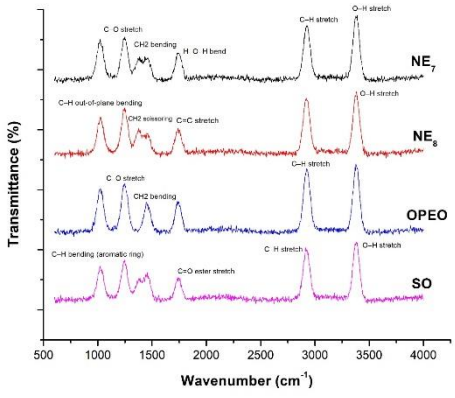


Figure 6. FTIR spectrum of stable treatments of nanoemulsion 1:4 (v/v) oil-to-surfactant ratios (a) NE_7 (b) NE_8

3.7. pH value

The pH is a very important parameter for assessing the stability of nanoemulsions, fluctuation in pH value indicates the presence of chemical reactions that may affect the quality of the final product. The pH of nanoemulsion treatments with different oil-to-surfactant ratios is presented in **Table 2**. All treatments of nanoemulsion showed pH value above 7 at 0 day and slight change had been observed at 20 days of storage period as shown in **Figure 7**. But at 40 days the pH value of various treatments was significantly reduced and a cloudy suspension of accumulated particles was made. Results revealed that accumulation of particles occurred nearly at 5.5 pH value, it diminishing the translucency of nanoemulsion and triggering phase separation. Similar findings have been recorded by Lee *et al.* (2011). The instability of nanoemulsion was associated with coalescence; it reduced the electrostatic repulsion of particles over a certain pH range, especially at lower pH values (Bai & McClements 2016).

Ren *et al.* (2021) evaluated the response of nanoemulsion at different pH conditions and summarized that conversion of pH value from basic to acidic conditions, nanoemulsion was disrupted within 30 mins due to coalescence phenomena. However, 1:4 (v/v) oil to surfactant ratio treatments NE_7 (7.10 to 6.01) and NE_8 (7.07 to 6.08) exhibited less change in pH value from 0 to 60 days of storage period among all treatments. Numerous instability processes of nanoemulsions including coalescence, flocculation, phase separation, creaming, Ostwald ripening and sedimentation are affected by various parameters, such as solubilization, temperature, heat treatment, ionic strength, particle charge, pH strength, and particle size distribution (Karthik *et al.* 2017).

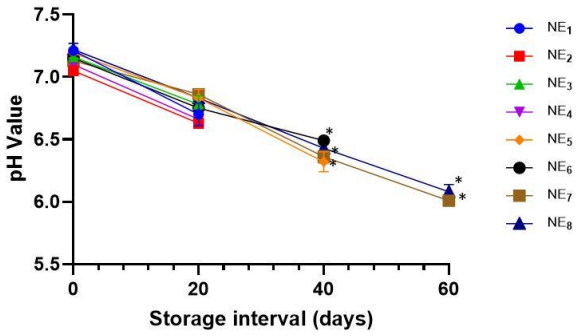


Figure 7. pH value of nanoemulsion during 60 days of storage interval at 25°C (Mean ± S.D)

3.8. Antioxidant activity

Nano size droplets boost the biological action of hydrophobic compounds as surface area per unit mass is enhanced (Chen *et al.* 2021). Polyphenols in essential oils have high antioxidant potential and reduce the excess of DPPH free radicals (McClements & Rao 2011). Oil and surfactant concentration are the main parameters that influence the nanoemulsion antioxidant activity. The antioxidant activity of nanoemulsion treatments with different oil-to-surface ratios is presented in **Table 2**. All

treatments of nanoemulsion showed above 50% antioxidant activity at 0 day and it remained quite stable till 20 days of the storage period as shown in **Figure 8**. But at 40 days the antioxidant activity of various treatments going to reduce, which might be due to larger particle size. The results revealed that a large amount of surfactant was useful for preventing the antioxidant activity of nanoemulsion by trapping the bioactive compounds (sesamolin, sesamin, and limonene) existing in SO and OPEO. Artiga-Artigas *et al.* (2018) also reported the same results that the highest free radical scavenging activity was observed at a high concentration of tween 20 surfactant. An excessive amount of surfactant can produce micelles; responsible for trapping the bioactive compounds, preserving the encapsulated curcumin and subsequently enhancing the antioxidant potential of the system (Ratanajiajaroen *et al.* 2012).

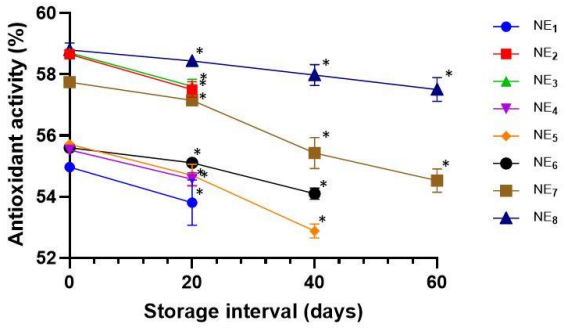


Figure 8. Antioxidant activity (%) of nanoemulsion during 60 days of storage interval at 25°C (Mean ± S.D)

Although the 1:4 (v/v) oil to surfactant ratio treatment NE_7 and NE_8 exhibited superior droplet size reduction and physical stability, they showed only slightly lower antioxidant activity compared to some mid-range formulations. For instance, NE_2 (1:1 v/v) had an antioxidant activity of 58.66% at day 0, while NE_8 1:4 showed 58.80%. This marginal difference may be attributed to the higher surfactant content in NE_8, leading to enhanced encapsulation of bioactive compounds, while stabilizing the emulsion, which may reduce their free availability to neutralize DPPH radical. Thus, a higher surfactant ratio may create a tighter interfacial layer that limits the immediate release or accessibility of antioxidant molecules into the aqueous phase. This indicates a trade-off between achieving smaller particle size and maintaining maximum antioxidant effectiveness, highlighting the need for optimized surfactant levels that balance stability and bioactivity. However, 1:4 (v/v) oil to surfactant ratio treatments NE_7 (57.74 to 54.53 %) and NE_8 (58.80 to 57.50 %) exhibited a slight change in antioxidant activity from 0 to 60 days of storage period among all treatments. The reduction indicates that the nanoemulsion maintained substantial antioxidant potential during prolonged storage. Such stability supports its functional applicability in food and pharmaceutical products where extended shelf life and retention of bioactivity are crucial. The encapsulation of OPEO in the nanoemulsion system likely protected the bioactive compounds from degradation caused by environmental factors such as light, oxygen, and temperature. Therefore, despite the slight reduction, the nanoemulsion still qualifies for functional use with health-promoting benefits.

Limitations and Future Prospects

While the study successfully optimized a stable OPEO-SO nanoemulsion using ultrasonic emulsification and demonstrated desirable physiochemical and antioxidant properties, it was conducted under controlled laboratory conditions. Real-world application trials in actual food systems, edible coatings, or pharmaceutical formulations were not performed. Additionally, only one type of surfactant (Tween 20) and carrier oil (SO) was evaluated, and sensory, morphological, or toxicological assessments were not included. These limitations should be addressed in future research to fully validate the commercial applicability and safety of the developed nanoemulsion in practical settings.

Conclusion

In this study, a stable OPEO nanoemulsion was developed using an energy-efficient ultrasonication technique. The optimized formulation, comprising 80% of OPEO and a 1:4 (v/v) oil-to-surfactant ratio, achieved a small particle size (83.66 nm), low PDI (0.24), and strong zeta potential (-31.93 mV), demonstrating excellent colloidal stability and antioxidant retention over 60 days of ambient storage. FTIR and SEM analyses further confirmed uniform molecular dispersion and consistent droplet morphology. These outcomes underscore the success of formulation optimization in enhancing the long-term physicochemical and functional performance of the nanoemulsion system. The current research focused primarily on structural stability and antioxidant characterization; therefore, antimicrobial activity and real food application trials were beyond the present scope. However, future studies will aim to validate these nanoemulsions in actual food matrices and evaluate their antimicrobial potential to support broader industrial applicability.

Conflict of Interest

The authors declare no conflicts of interest

Author Contributions

A.S. and M.A.R.: provided the conceptualization, A.S.: finalized the methodology, A.S., A.H., and M.S.: validation, visualization, and software, A.S.: wrote the original draft and performed the analysis, A.S., M.A.R., A.H., and M.S., A.S, M.A.R.: review and editing, M.A.R.: supervision and project administration, HEC provided the funding to Indigenous scholar A.S. (Pin No. 520-141270-2AV6-86). All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data used to support the findings of this study can be made available by the corresponding author upon request.

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