International Journal of Nanoelectronics and Materials
IJNeaM
ISSN 1985-5761 | E-ISSN 2232-1535

Formulation and Characterization of Lemon Myrtle Essential Oil in Water Nanoemulsion Prepared Via Ultrasonication

Siti Noraini Bunawan[a,b], Noor Azlina Masdor[a], Nur Adeela Yasid[b], Mohd Izuan Effendi Halmi[c], Raviraj Mahabaleshwar Kulkarni[d], and Mohd Yunus Abd Shukor[b,*]

[a]*Agri-nanotechnology Programme, Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute, 43400 Serdang, Selangor, Malaysia*
[b]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[c]*Department of Soil* Management, *Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[d]*Department of Chemistry, School of Computing, MIT Vishwaprayag University, Solapur- 413255, India*
**Corresponding author. Tel.: +6-03-97696722; e-mail: mohdyunus@upm.edu.my*

Received 23 June 2025, Revised 24 Nov 2025, Accepted 21 May 2026

ABSTRACT

This study focused on the formulation and characterization of a lemon myrtle essential oil-in-water nanoemulsion prepared via ultrasonication. The effects of key process parameters, specifically sonication power amplitude, sonication time, and surfactant concentration, were optimized based on droplet size, polydispersity index (PDI), and the stability of the nanoemulsion. Optimization of the formulation was achieved at 100% sonication power for 5 min, using 1% (w/w) lemon myrtle essential oil and 4% (w/w) surfactant, resulting in a droplet size of approximately 12.52 nm and a PDI of 0.153. Further characterization included physical appearance, droplet distribution, zeta potential, morphology, Fourier-transform infrared spectroscopy (FTIR), and storage stability. A clear nanoemulsion was obtained with a zeta potential of -38.1 mV, and transmission electron microscopy (TEM) revealed a spherical morphology. FTIR analysis confirmed that no chemical changes occurred in the components of lemon myrtle essential oil after ultrasonication. The nanoemulsion remained physically stable at both 25°C and 4°C for up to 180 days. In this work, a stable lemon myrtle essential oil-in-water nanoemulsion was successfully developed via ultrasonication-assisted processing. The developed nanoemulsion has promising applications in industries such as food, agriculture, and pharmaceuticals.

Keywords: *Essential oil, lemon myrtle, oil-in-water nanoemulsion, ultrasonication*

1. INTRODUCTION

Essential oils (EOs) extracted from aromatic plants have garnered considerable interest due to their natural and diverse biological properties, including antimicrobial, anti-inflammatory, and antioxidant activities. Lemon myrtle (*Backhousia citriodora*) is an indigenous Australian native herb. Its leaves are a rich source of EO, which is extracted primarily via steam distillation. Lemon myrtle EO is notable for its pleasant fragrance and high citral content, typically comprising over 90% of the oil. Citral comprises two stereoisomers, geranial and neral. Both isomers exhibit strong antibacterial, antifungal, antioxidant, and anti-inflammatory activities [1]–[3], making them promising candidates for various applications, including pharmaceuticals and the food and agricultural industries.

Lemon myrtle EO is increasingly acknowledged as a high-value crop with outstanding commercial export potential. As with other EOs, issues such as low water solubility, volatility, and susceptibility to oxidative degradation have limited the stability and shelf life of lemon myrtle EO in product formulations. To address these limitations, incorporating lemon myrtle EO into nanoemulsion systems is being explored as a viable strategy to enhance its dispersion in water, increase bioavailability, and protect its bioactive constituents from degradation [1].

Nanoemulsion stands out as the most accessible, inexpensive, and user-friendly among nanocarrier approaches for EO [2]. Nanoemulsions require minimal amounts of surfactant [3], usually less than 10% compared to nearly 15% in macroemulsions, as well as the low surfactant-to-oil ratio (SOR) required for their formation [4]. EOs, insecticides, and other substances with poor water solubility or lipophilicity can be effectively dispersed in aqueous media using nanoemulsion [5,6]. The physicochemical qualities and stability of EOs can also be enhanced by a nanoemulsion approach, which reduces their volatility and shields them from environmental interactions. The advantages of EO nanoemulsions are not only associated with improved physicochemical stability but also with enhanced bioavailability [2,7]. As a result, nanoemulsified EOs often exhibit enhanced bioactivity compared to their free or bulk forms, thus making them more attractive. For instance, Mukurumbira et al. [8] reported that lipid-based nanoemulsions exhibited greater antimicrobial activity than oils in microemulsion or bulk form. Studies on thymol nanoemulsion have shown vigorous antibacterial activity compared to bulk thymol,

which exhibited negligible effect, demonstrating the superior antimicrobial efficacy of the nanoemulsified form [16]. This observation supported the common observations that lemon myrtle essential oil in the form of nanoemulsions may exhibit enhanced biofunctional activity relative to the native EO, further reinforcing its prospective industrial and pharmaceutical significance.

Ultrasonication is an approach that utilizes acoustic cavitation to produce fine droplets and uniformly dispersed nanoemulsions, thereby conserving the integrity and activity of bioactive compounds [9]. Ultrasonication allows precise control over formulation parameters, for example, energy input, surfactant concentration, and droplet size, which can be optimized for specific applications of the nanoemulsion. For this reason, ultrasonication is recognized not only as an effective method for preparing EO nanoemulsions but also as a simple and economical one [10]. The stability and functional bioactivity of EOs could be improved by formulating them into a nanoemulsion via ultrasonication, thereby making EOs more suitable for a wide range of applications [11,12].

The study aims to formulate and characterize a stable oil-in-water nanoemulsion containing lemon myrtle EO and evaluate its physicochemical properties. This study employed high-energy ultrasonication to prepare a lemon myrtle EO nanoemulsion. The influence of ultrasonic process parameters (power and amplitude, and sonication time) and the surfactant-to-oil ratio was investigated to develop the nanoformulation. The physicochemical characteristics of the lemon myrtle EO nanoemulsion were characterized, including droplet size, polydispersity index (PDI), optical properties (visual appearance and transmittance), and nanoemulsion stability.

2. MATERIALS AND METHODS

2.1. Lemon Myrtle EO and Surfactant

The 100% pure lemon myrtle EO was used in the study. The lemon myrtle EO was distilled from lemon myrtle leaves (*Backhousia citriodora*) grown at the Malaysian Agricultural Research and Development Institute (MARDI) in Kuala Linggi, Negeri Sembilan, Malaysia. Both non-ionic surfactant mixtures, Tween 80 and Span 80, were purchased from Sigma-Aldrich (USA). All experiments were conducted using water filtered through a Milli-Q system (Millipore Co., Bedford, MA, USA) with 0.2 μm filters.

2.2. Preparation of Nanoemulsion

The study employed Tween 80 and Span 80 as a surfactant mixture with an HLB of 14, which had been previously optimized [13]. The nanoemulsion was formed by vortexing an organic phase comprising a surfactant and lemon myrtle with pure double-distilled water, which served as the aqueous phase. The components of the emulsion were quantified in weight percentages using an analytical balance. After that, the organic and water phases were combined by vortexing at 2000 rpm for 2 min to create a coarse emulsion [14]. Next, the coarse emulsion was sonicated using a high-intensity ultrasonic processor (Q125 Qsonica Sonicator, USA) capable of generating 125 W at 20 kHz to produce a fine emulsion. A titanium alloy microtip probe with a diameter of 3 mm was affixed to the sonicator. The sonicator probe was placed 20 mm from the bottom surface of the bottle. To avoid sample heating, sonication was performed with 5 s ON and 9 s OFF.

2.3. Formulation Optimization

2.3.1. Sonication power amplitude

To optimize sonication amplitude, the formulation composition was set to 1% (w/w) lemon myrtle EO, 1% (w/w) surfactant, and 98% (w/w) water. The formulation was sonicated for 1 min at different power levels. The input sonicator power levels were set to 50%, 75%, and 100% of the total input power (125 W), corresponding to 63 W, 94 W, and 125 W, respectively [15]. The experiments were prepared in triplicate. After 24 h at room temperature, nanoemulsion samples were characterized for droplet size and PDI measurements, macroscopic appearance and turbidity measurements, and centrifugation stability testing.

2.3.2. Sonication time

For sonication time optimization, the formulation composition was set to 1% (w/w) lemon myrtle EO, 1% (w/w) surfactant, and 98% (w/w) water. Various sonication times (1, 3, 5, and 7 min) were employed. Samples were then sonicated at 100% amplitude and characterized after 24 hours of preparation. The experiments were prepared in triplicate.

2.3.3. Surfactant concentration

The surfactant was optimized from 1% to 5% (w/w) with 1% (w/w) lemon myrtle EO. The water compositions also varied (from 94% to 98%) depending on the surfactant composition. The samples were then sonicated at the optimal sonication time and amplitude. The experiments were prepared in triplicate. The samples were then characterized after 24 hours of preparation.

2.4. Lemon Myrtle Nanoemulsion Characterization

2.4.1. Droplet size, polydispersity index, and zeta potential measurement

The NanoBrook Omni particle analyzer from Brookhaven Instruments, UK, was used to measure the size of the nanoemulsion droplets, the polydispersity index (PDI), and the zeta potential of the lemon myrtle EO nanoemulsion formulation. The droplet size and PDI were quantified using dynamic light scattering (DLS). Meanwhile, phase analysis light scattering (PALS) was used to determine the zeta potential using laser Doppler electrophoresis. A 1:20 (v/v) ratio of ultrapure deionized water to a nanoemulsion sample was used to minimize scattering effects. The temperature and scattering angle used for the

measurements were 25°C and 90°, respectively [16]. Measurements were performed in triplicate, and the data were presented as mean values.

2.4.2. Macroscopic appearance and turbidity measurement

A UV-Vis spectrophotometer (OPTIZEN POP, K Lab, South Korea) was used to quantify the turbidity of the resulting nanoemulsions. The measurement was performed by calculating the transmittance (%T) of the undiluted nanoemulsion sample at 600 nm [17]. A standard distilled water solution was used for the analysis. The light transmission of the reference beam across the samples was quantified, and the corresponding transmittance percentages were recorded [18].

2.4.3. Centrifugation stability test

Centrifugation was used as an early screening method for the stability of the nanoemulsion sample. The samples were centrifuged for 30 min at 5000 rpm to check for any phase separation. The absence of phase separation indicates a stable nanoemulsion formulation.

2.4.4. Transmission electron microscopy

Lemon myrtle EO nanoemulsion droplet samples were mixed with the negative stain (methylamine tungstate) in a 1:1 ratio. The mixture was then dropped onto a Lacey formvar carbon film supported on a copper grid (ProSciTech Pty Ltd, Australia). The samples were then dried by evaporation at room temperature. The Hitachi TEM system (Hitachi High-Tech, Japan) was used to study the morphology of nanoemulsion droplets at 120 kV.

2.4.5. Fourier Transform Infra-red (FTIR) analysis

Interactions between each component in the nanoemulsion were determined via FTIR analysis using an attenuated total reflection (ATR) unit. Measurement was carried out over the 400–4500 cm^{-1} wavenumber range using an FTIR spectrophotometer (Spectrum 100, PerkinElmer, USA) equipped with an ATR cell. The interactions between lemon myrtle EO, surfactant, and distilled water that were involved in the lemon myrtle EO nanoemulsion formation were observed.

2.4.6. Storage stability test

The selected lemon myrtle EO nanoemulsion formulation was stored at 4°C, 25°C, and 40°C. Stability was measured over an 180-day storage period and assessed by determining the mean droplet size and PDI. The nanoemulsion samples were also visually inspected for changes, such as creaming and phase separation [14,19].

2.4.7. Statistical analysis

Statistical analysis was conducted using one-way ANOVA via GraphPad Prism Software Version 10.4.1. A p-value less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1. Formulation of Nanoemulsion

3.1.1. Optimization of sonication power amplitude

The amplitude of the sonication power is one of the process parameters that can influence the characteristics of nanoemulsion droplets. In this study, lemon myrtle EO emulsions with various inputs of power amplitude were prepared with a composition of 1% (w/w) of lemon myrtle oil, 1% (w/w) of surfactant, and 98% (w/w) of water with 1 min of sonication time. Figure 1 shows that the mean diameter of the lemon myrtle EO nanoemulsion droplet size decreased with an increase in the power of the amplitude, ranging from 50 to 100%. The minimum lemon myrtle EO nanoemulsion droplet size decreased to 60 nm at 100% sonication amplitude, compared to 80 nm and 126 nm at 75% and 50% power amplitude, respectively. An increase in amplitude increased shear stress, as more energy was dissipated into the system [9,15]. The decrease can be explained by the fact that a large number of tiny cavitation bubbles collapsed rapidly, resulting in a smaller droplet size of lemon myrtle oil. Other studies also reported similar findings. For example, the droplet size of the mustard oil nanoemulsion decreased as the power amplitude increased from 20% to 40% [20]. As the intensity of the ultrasonic field increased, the average droplet size in the clove oil nanoemulsion decreased [15]. Alderees et al. [1] reported that Tasmanian pepper leaf EO, anise myrtle EO, and lemon myrtle EO had significantly reduced their nanoemulsion droplet size and PDI with an increase in sonication amplitude.

An increase in power amplitude also affects the PDI value. Based on Figure 1, the PDI value slightly decreased from 0.278 at 50% sonication amplitude to 0.245 at 100% sonication amplitude. A lower PDI indicates a narrower particle size distribution, resulting in more uniform droplet sizes [21].

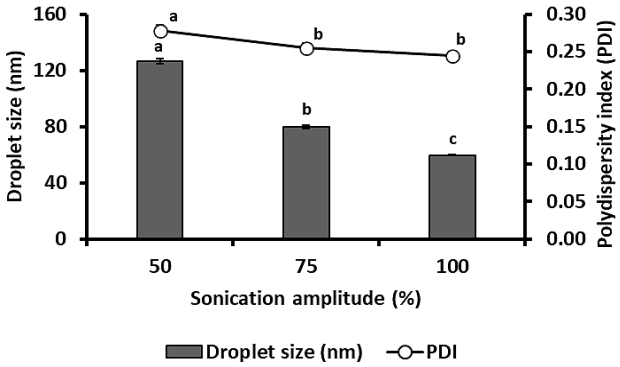

Figure 1. Effects of different sonication amplitudes (50%, 75%, and 100%) on droplet size and polydispersity index (PDI) of lemon myrtle essential oil (EO) nanoemulsions prepared using the ultrasonication method. Results are expressed as mean ± SD (n = 3). Different letters above the bars indicate significant differences between treatments at $p < 0.05$ (one-way ANOVA). Higher amplitude significantly reduced droplet size and improved uniformity (lower PDI).

The optical properties of the nanoemulsion were also affected by the increasing amplitude (Figure 2). The stability of EO nanoemulsion corresponds to its integrity, which is closely related to particle size [17] since the increasing amplitude has decreased size, therefore, to the lesaurbid lemon myrtle EO nanoemulsion solution from 2% T at 50% sonication amplitude to 33% T at 100% sonication amplitude (Figure 3).

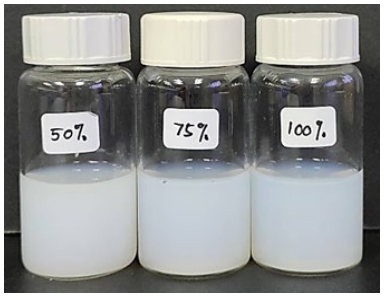

Figure 2. Visual appearance of lemon myrtle EO nanoemulsions prepared at different sonication amplitudes (50%, 75%, and 100%). Samples became less turbid as amplitude increased, reflecting smaller droplet size and improved optical clarity.

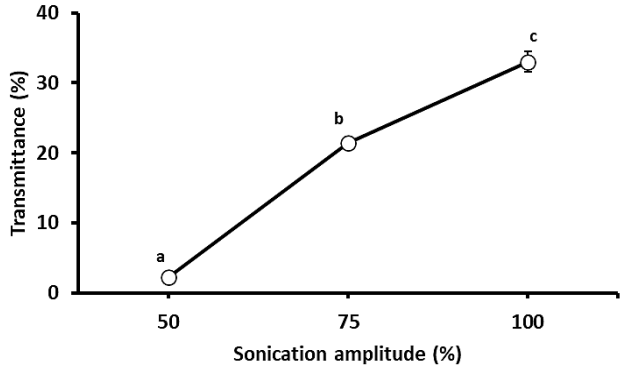

Figure 3. Effect of sonication amplitude on turbidity (% transmittance at 600 nm) of lemon myrtle EO nanoemulsions prepared by ultrasonication. Experimental measurements are expressed as mean ± SD (n = 3). Higher amplitude yielded significantly clearer nanoemulsions, as indicated by increased % transmittance values. Different letters indicate significant differences at $p < 0.05$.

It is necessary to evaluate the stability of nanoemulsions, and one way to do this is by a centrifugation test to predict their long-term stability. Nanoemulsion formulation with no phase separation after the centrifugation test indicates a stable nanoemulsion, while a nanoemulsion with phase separation indicates instability of the formulation. Table 1 presents the centrifugation test results for lemon myrtle nanoemulsions at different sonication power levels. The nanoemulsion formulations exhibited instability, characterized by phase separation. Centrifugation can accelerate both sedimentation and creaming. This demonstrates that the emulsion rupture is likely linked to the gravitational force [20,22]. These unstable nanoemulsions indicate that the formulations proposed in Table 1 were not stable and require optimization with respect to another parameter.

Table 1 Centrifugation test results and visual appearance of nanoemulsion samples at different sonication amplitudes

Fixed parameter	Amplitude	Centrifugation test	Visual appearance
1% EO 1% surfactant 1 min sonication	50%	Unstable with phase separation	Cloudy
	75%	Unstable with phase separation	Cloudy
	100%	Unstable with phase separation	Cloudy

3.1.2. Optimization of sonication time

The next important process parameter in the ultrasonication method that impacts the characteristics of the nanoemulsion droplet is the sonication time. The time it takes to sonicate has a significant impact on the droplets' size. A longer nonoptimal sonication time exposed the emulsion to the turbulent acoustic field conditions [20]. Proper adjustment of the ultrasonication time enhanced the dispersion state and improved the stability of the nanoemulsion [9]. The droplets may become more deformed and fragmented during prolonged sonication as the shear forces on them increase. It is anticipated that extended sonication times will result in reduced droplet sizes [15]. Figure 4 shows the effect of lemon myrtle EO nanoemulsions produced by ultrasonication under varying sonication times. There was a substantial decrease in droplet size from 1 to 5 minutes ($p < 0.05$); however, this decrease did not persist from 5 to 7 minutes. These were due to the surfactant's ability and effectiveness, which determined how much the droplet size decreased. As long as there is sufficient emulsifier to surround the newly formed droplets fully, the droplet size decreases as the energy used to homogenize the mixture increases [14].

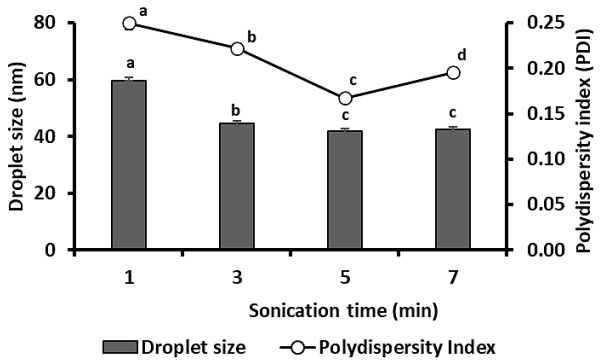

Figure 4. Effects of sonication time (1, 3, 5, and 7 minutes at 100% amplitude) on droplet size and PDI of lemon myrtle EO nanoemulsions. Experimental measurements are expressed as mean ± SD (n = 3). Different letters above bars denote significant differences ($p < 0.05$). Droplet size decreased with the extended sonication time up to 5 minutes, with no further improvement at 7 minutes.

Prior research has reported parallel outcomes. Nirmal et al. [14] observed that the clove EO droplet size was dramatically reduced by probe sonication for 1–5 minutes, with no discernible effect observed with longer sonication durations. Carpenter and Saharan [20] found that after 30 minutes of sonication, the average droplet size of the mustard oil nanoemulsion decreased steadily from 176.1 nm to 87.38 nm as the sonication duration increased. Furthermore, Liu et al. [23] discovered that a 1 min ultrasonication treatment successfully reduced the size of the ginger EO nanoemulsion droplet. Even after sonicating the ginger nanoemulsion for 10 min, its size remained unchanged.

In addition, the distribution of droplet sizes is controlled by cavitational effects. The PDI decreased from 0.250 to 0.167 as the sonication duration increased from 1 to 5 min (Figure 4). These results indicate that the droplet size distribution became more uniform with increasing sonication duration, as reported in the literature [20]. However, a prolonged 7-min ultrasonication time increased the PDI.

Visual observation (Figure 5) shows that the proposed nanoemulsion formulations were still cloudy. However, the formulations showed an increase in transmittance percentage from 33 to 42% T when the duration of the sonication process was extended from 1 to 5 min ($p < 0.05$) (Figure 6). After 5 minutes of sonication, the transmittance percentage increased no further.

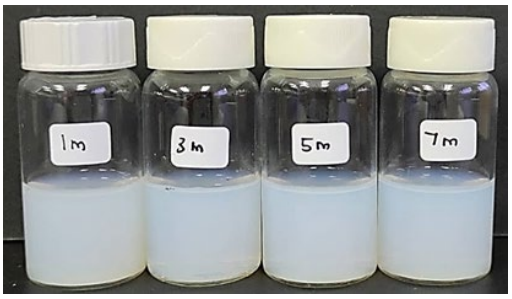

Figure 5. Visual appearance of lemon myrtle EO nanoemulsions prepared at different sonication times (1, 3, 5, and 7 minutes). Formulations appeared progressively clearer with longer sonication up to 5 minutes.

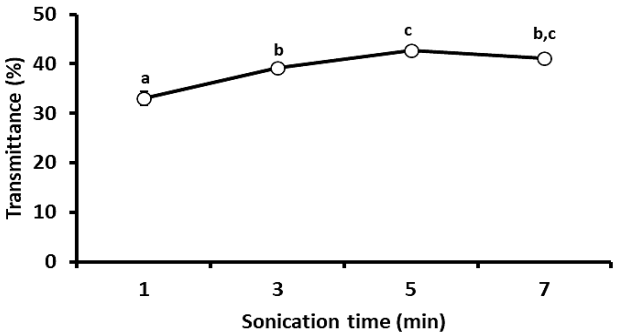

Figure 6. Effect of sonication time on turbidity (% transmittance at 600 nm) of lemon myrtle EO nanoemulsions. Results are expressed as mean ± SD (n = 3). Increased sonication time led to significantly higher % transmittance values up to 5 minutes, consistent with smaller droplet sizes. Different letters indicate significant differences at $p < 0.05$.

Table 2 Centrifugation test results and visual appearance of nanoemulsion samples at different sonication times

Fixed parameter	Sonication time (min)	Centrifugation test	Visual appearance
1% EO 1% surfactant 100% amplitude	1	Unstable with phase separation	Cloudy
	3	Unstable with phase separation	Cloudy
	5	Unstable with phase separation	Cloudy
	7	Unstable with phase separation	Cloudy

Based on the influence of sonication time on droplet size, PDI, and transmittance in nanoemulsion formulations, it was found that ultrasonication was inefficient for cavitation, as it caused macro turbulence from microbubble implosion and microjets in the lemon myrtle EO after 5 min. As a result, it was determined that the sonication time during the experiment should be set at 5 min.

All the nanoemulsion formulations were subjected to stability assessment via a centrifugation test. Although the formulations were in nano size (below 100 nm), they were unstable after the centrifugation test (showing phase separation) (Table 2), which might be due to the insufficient amount of surfactant.

3.1.3. Optimization of surfactant ratio

Increasing the sonication power and duration reduces the energy required to break up oil droplets and decreases the surface tension at the oil-water interface. Hence, a substantial proportion of surfactant is needed to coat the droplet's surface adequately. Therefore, to optimize the optimum surfactant needed for lemon myrtle EO nanoemulsions, different surfactant concentrations (1- 5%) were prepared with a constant of 1% EO, and 5 min ultrasonication time at 100% amplitude.

Figure 7 shows the effects of surfactant concentration on droplet size and PDI. The increase in surfactant concentration has decreased the mean droplet size of the lemon myrtle nanoemulsion from 45.5 nm to 12.52 nm. A substantial amount is required to coat the droplet surface adequately. An increased surfactant concentration can reduce the mean droplet size by promoting surfactant adsorption at the interfacial membrane. This condition facilitates the creation of smaller droplets. An EO nanoemulsion is more stable when its droplets are smaller in size [21]. Based on the results, the economical surfactant concentration that produced the smallest nanoemulsion droplets of lemon myrtle EO was 4%.

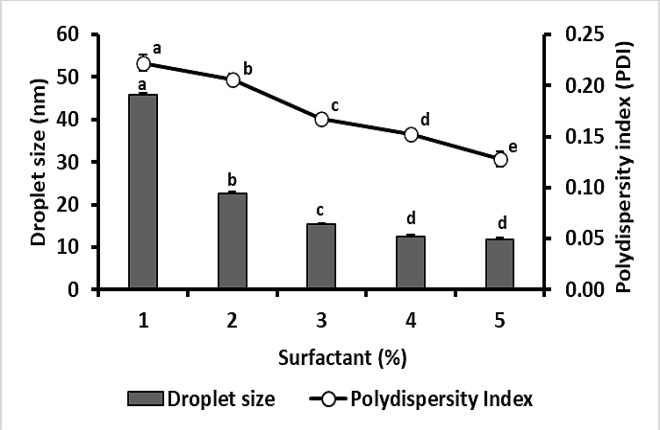

Figure 7. Effects of surfactant concentration (1–5% w/w) on droplet size and PDI of lemon myrtle EO nanoemulsions prepared at 100% amplitude for 5 minutes. Results are expressed as mean ± SD (n = 3). Different letters indicate significant differences between treatments ($p < 0.05$). Increasing surfactant concentration reduced droplet size and produced more uniform dispersions (lower PDI).

In the present study, the lowest PDI value (0.128) was obtained by the nanoemulsion formulated with 5% surfactant. The PDI of lemon myrtle EO nanoemulsion decreased from 0.226 to 0.128 with the increase in surfactant concentration (Figure 7), indicating that a more uniform distribution of lemon myrtle EO oil droplets was produced.

Turbidity is a multifaceted characteristic influenced by several parameters, including the oil's composition, mean droplet size, particle concentration, and PDI [18]. In the present study, increasing the surfactant concentration reduced the turbidity of the lemon myrtle EO nanoemulsion. The nanoemulsion formulations (Figure 8) became less turbid and more optically transparent as surfactant concentration increased. The transmittance percentage was increased from 35.9% T at 1% surfactant to 98.8% T at 5% surfactant concentration (Figure 9). Liew et al. [18] suggested that a transparent and clear appearance indicates that small particles in the nanometre range were produced. As the size of the droplets decreases, the transmittance measurement increases. Nanoemulsion transparency, droplet size reduction, and interfacial tension lowering result from an increase in surfactant concentration. Nanoemulsions have droplet sizes smaller than the wavelengths of visible light, as indicated by their optically transparent/clear formula [24]. Consistent results were also reported by Liew et al. [18], who noted that different types of lime EO showed different transmittance percentage readings due to differences in mean droplet sizes. The percent transmittance of lime EO was 78% for calamansi lime, 96% for kaffir lime, and 99% for key lime, with mean droplet sizes of 60 nm, 28 nm, and 21 nm, respectively.

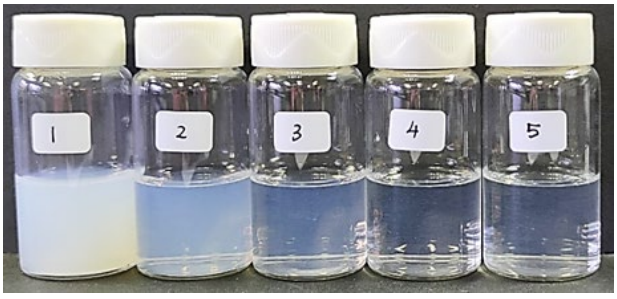

Figure 8. Photographic images of lemon myrtle EO nanoemulsions prepared with increasing surfactant concentrations (1–5% w/w). Samples transitioned from a cloudy/milky appearance to an optically transparent state as surfactant concentration increased.

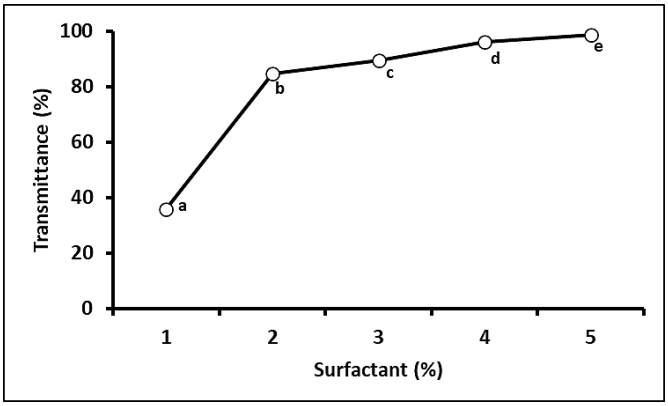

Figure 9. Influence of surfactant concentration (1–5% w/w) on turbidity (% transmittance at 600 nm) of lemon myrtle EO nanoemulsions. Results are expressed as mean ± SD (n = 3). Higher surfactant concentration significantly increased % transmittance, reflecting clearer emulsions and smaller droplet sizes.

The clear visual feature is a common characteristic of nanoemulsions, in which any increase in turbidity can be associated with an increase in the size of the nanodroplets and subsequent destabilization [25]. All the nanoemulsion samples were tested for fast-screen stability using a centrifugation test. Lemon myrtle EO nanoemulsions formulated with 3 to 5% of surfactant have a stable nanoemulsion, which did not show any phase separation after the centrifugation test (Table 3).

Centrifugation can accelerate the instability process in nanoemulsions by applying gravitational force [22]. With sufficient surfactants present, steric hindrance prevents droplets from repelling one another, and ultrasonication reduces droplet size and improves droplet uniformity. The synergistic effect of ultrasonication and sufficient surfactant helps preserve the stability of the nanoemulsion under the shear forces of a centrifuge [20]. Christy et al. [24] screened the stable oil-in-water nanoemulsion formulation based on the acquired transmittance percentage data. The formulation that exhibits over 95% transmittance was chosen as the preferred formulation because it indicates high stability. In this study, the lemon myrtle EO nanoemulsion containing 4% and 5% surfactants exhibits a percent transmittance above 95% (Figure 9). Lemon myrtle EO nanoemulsion formulated with 4% surfactant has a transmittance measurement of 96% T, while nanoemulsion formulated with 5% surfactant has a transmittance measurement of 98% T.

Table 3 Centrifugation test results and visual appearance of nanoemulsion samples at different sonication times.

Fixed parameters	Surfactant (%)	Centrifugation test	Visual Appearance
1% EO 100% amplitude 5 min sonication	1	Unstable with phase separation	Milky
	2	Unstable with phase separation	Cloudy
	3	Stable, no phase separation	Translucent
	4	Stable, no phase separation	Clear
	5	Stable, no phase separation	Clear

Based on the results obtained, lemon myrtle EO nanoemulsions formulated with 4% and 5% surfactant concentrations met the desired formulation criteria, including small droplet size, low PDI (below 0.2), high optical transparency (transmittance above 95%), and good stability. However, considering economic factors, the lemon myrtle EO nanoemulsion formulated with a 4% surfactant concentration was selected as the optimal formulation in the present study.

3.2. Characterization of Nanoemulsion

3.2.1. Droplet size and its distribution

Figure 10 presents the dynamic light scattering (DLS) results for the PDI measurement of the lemon myrtle EO nanoemulsion prepared using the optimized formulation. The droplet size showed a uniform distribution, as indicated by a single monomodal peak in the PDI measurement. The droplet sizes ranged from 4.0–43.5 nm, with an average diameter of 12.52 nm and a PDI value of 0.128, suggesting that the lemon myrtle EO nanoemulsion exhibited high uniformity and stability.

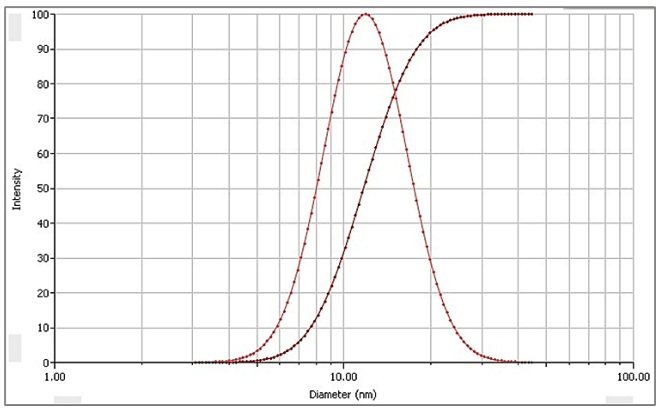

Figure 10. Droplet size distribution of lemon myrtle EO nanoemulsion prepared using the optimized formulation (1% EO, 4% surfactant, 100% amplitude, 5 min). Measurement was performed by dynamic light scattering (DLS). A monomodal distribution was observed with an average droplet diameter of 12.52 nm and PDI of 0.128, indicating uniform dispersion.

A smaller PDI value indicates a more homogeneous dispersion of nanoemulsion droplets. A PDI value below 0.2 indicates a good uniformity of EO droplet size [4,21]. A study by Nirmal et al. [13] obtained lemon myrtle nanoemulsion droplets with a size of 16.07 nm with a PDI of 0.209 using a nanoemulsion formulation composed of 1% lemon myrtle EO, 10% surfactant, and 89% water. In addition, consistent with our results, Azmi et al. [26] reported that an ultrasonically prepared oil-in-water nanoemulsion containing lemon oil not only improves physical stability but also enhances functional bioactivity, supporting the potential application of lemon myrtle EO nanoemulsion beyond physicochemical characterization.

3.2.2. Zeta potential

Figure 11 shows the results of a phase analysis light scattering (PALS) measurement of the zeta potential of a nanoemulsion of lemon myrtle EO made with the optimized formulation. The stability of an emulsion system can be predicted and controlled using the measurable characteristic known as zeta potential. An emulsion is said to be stable when it has a zeta potential value greater than 30. However, an unstable emulsion is indicated by a score of 30 or below. The pH can be represented by a positive (+) or negative (-) value; a low pH indicates an acidic medium, whereas a high pH indicates an alkaline one [27]. To obtain accurate findings from a PALS test, the particles need only travel a small fraction of their diameter [28]. In this study, the zeta potential was -38.1 mV. The optimized formulation yielded stable nanoemulsion droplets ranging in size from nanometers, as shown by this data. Another indication of the nanoemulsion's strong energy barrier for dispersed droplet coalescence is the zeta potential value [29].

Tween 80 and Span 80, two non-ionic surfactants, were employed to coat the lemon myrtle EO, which is supposed to be chargeless. Unfortunately, a negative charge of -38.1 mV was observed. Possible causes of this effect include the interaction of the polyoxyethylene group with water, as well as the selective adsorption of anions (-OH). The existence of an oxygen atom in both EO and surfactant mixture induces a change in the electrostatic double layer at the surface of nanoemulsion droplets [29]. The negative charge of the EO nanoemulsion is attributed to the free hydroxyl and carboxyl groups that can be released by various mechanical stressors, as reported [30,31]. Droplet surface charge, or zeta potential, can affect nanoemulsion stabilization by electrostatic repulsion. However, zeta potential is not considered an essential criterion for selecting the best formulation when non-ionic surfactants are used, as stability is primarily due to steric rather than electrostatic stabilization [27].

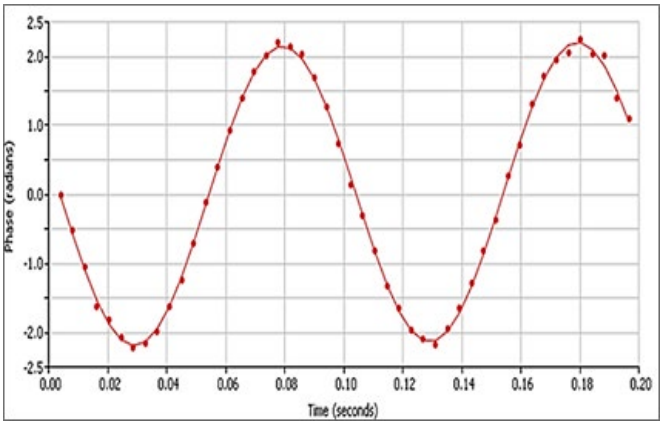

Figure 11. Zeta potential profile of optimized lemon myrtle EO nanoemulsion measured by phase analysis light scattering (PALS). The nanoemulsion exhibited a zeta potential of –38.1 mV, indicating strong repulsive forces between droplets and stable dispersion.

3.2.3. TEM view

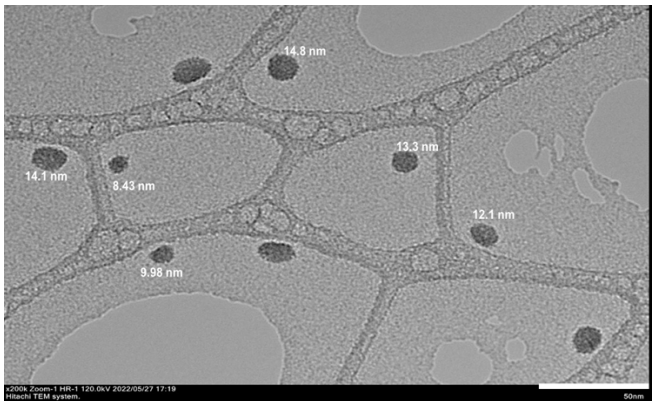

Figure 12. Transmission electron microscopy (TEM) image of lemon myrtle EO nanoemulsion droplets prepared with the optimized formulation. Spherical droplets with uniform morphology were observed, consistent with the DLS results.

An essential component of a nanoemulsion system was the droplet shape. The shape of nanoemulsion droplets was observed by images captured by a Transmission Electron Microscope (TEM). Based on the characterization by TEM view using Lacey formvar on the TEM copper grid, it can be inferred that lemon myrtle EO nanoemulsion's droplets were spherical in shape and in the nano size range (Figure 12). The results corroborate the findings of the DLS test, which indicated that the droplets were of consistent size and shape. The homogenization of the nanoemulsion and the effective blending of the surfactant and oil are shown by the spherical droplet form [32].

3.2.4. Fourier-transform Infrared Spectroscopy (FTIR) analysis

The oil phase may undergo chemical deformation as a result of cavitation and high pressure during sonication-induced emulsification. Acoustic cavitation, induced by ultrasonication, produces reactive free radicals by causing molecules to dissociate under the severe conditions of cavity collapse. This group of radicals can oxidize lipids [20]. Chemat et al. [33] found that the sunflower oil was oxidized due to the presence of free radicals produced by the ultrasonication procedure.

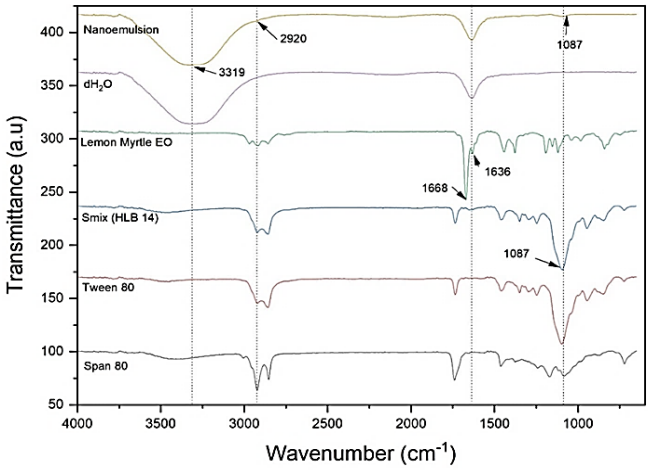

Figure 13. Fourier-transform infrared (FTIR) spectra of lemon myrtle EO, surfactants (Tween 80, Span 80), surfactant mixture (HLB 14), distilled water, and optimized nanoemulsion formulation. No new peaks were detected in the nanoemulsion compared to its components, indicating that ultrasonication did not induce chemical modifications in EO.

In light of this, the change in the chemistry of the lemon myrtle EO nanoemulsion was then analyzed using FTIR spectra for each component separately. A look at the FTIR spectra of Span 80, Tween 80, surfactant-mixture (smix) at HLB14, lemon myrtle EO, distilled water, and the nanoemulsion of lemon myrtle EO is shown in Figure 13. The stretching vibration of O-H bonds in H2O can be observed at 3319 cm^{-1}, as reported in lemon myrtle EO nanoemulsion [20,32]. The peak at 2920 cm^{-1} is typically associated with the stretching vibration of C-H bonds in aliphatic hydrocarbons, including terpenes, aldehydes, and alcohols, which are often found in large quantities in oils [32,34] and may contribute to peaks in different regions of the FTIR spectrum. Lemon myrtle EO is composed of terpenes, aldehydes, and alcohols as its major compounds [35]. The peak at 2920 cm^{-1} can probably be attributed to Tween 80 and Span 80, corresponding to their aliphatic hydrocarbon residues, which are nonpolar [36]. The 1087 cm^{-1} region in the FTIR spectrum of Tween 80 and span 80 could potentially correspond to several functional groups or

vibrations, including C-O stretching vibrations in ester groups, C-O-C stretching vibrations in ether groups, and C-C stretching vibrations in aliphatic chains. Peak at 1636 cm^{-1} in lemon myrtle EO typically relates to the stretching vibration of the carbonyl group (C=O) in aldehydes, ketones, or carboxylic acids [34]. The peak might also be interrelated with polyunsaturated fatty acids in the oil [32].

In conclusion, no extra specific peak was evident in lemon myrtle EO nanoemulsion FTIR analysis, thus specifying that lemon myrtle EO was stable and simply entrapped within Tween 80 and Span 80 surfactant mixture. Other researchers have also reported similar findings. Rao et al. [32] had observed no chemical changes during the preparation of rosehip oil nanoemulsion using probe ultrasonication. Carpenter and Saharan [20] additionally noted that the synthesis of mustard oil nanoemulsion did not include the occurrence of any additional peaks, thus proving that the emulsification process did not involve any chemical modifications brought about by ultrasonication. Shahavi et al. [16] also observed comparable outcomes when thymol nanoemulsion was generated via ultrasonication. This indicates that the emulsion was ultrasonically prepared by inducing solely physical effects.

3.2.5. Storage stability

Storage stability is paramount for the industrial application of EO nanoemulsions. Long-term physical stability can be assessed by observing changes in the mean droplet size at different storage temperatures [7, 13, 20]. Since the stability of oil-water-surfactant emulsion is temperature-dependent, this study investigated the effect of temperature on lemon myrtle EO nanoemulsion stability over time.

The selected nanoemulsion formulation was stored at 4°C, 25°C, and 40°C. The resulting droplet size variations are shown in Figure 3-14. The mean droplet size remained stable for 180 days at both 4°C and 25°C. On the other hand, storing the nanoemulsion at 40°C led to rapid instability, as evidenced by an increase in droplet size from 12 nm to 72 nm within the first 20 days. The increase reached 192 nm after 100 days of storage at this temperature.

These results validate the influence of temperature on droplet size and associated PDI value. Over a period of 180 days, the PDI value of lemon myrtle nanoemulsion stored at 25°C and 4°C was maintained below 0.173 (Figure 15), which indicates that the size distribution of the droplets in the lemon myrtle nanoemulsion was uniform, and these results were in line with the mean droplet size results. Meanwhile, at 40°C, the PDI value began to increase after 60 days, rising from 0.139 to 0.257 by 100 days. An increase in PDI indicates a change in the uniformity of nanoemulsion droplets and is also a sign of instability.

The destabilization of nanoemulsions results from Ostwald ripening, which causes droplets to grow during storage [17,29]. Ostwald ripening occurs when oil molecules diffuse across an aqueous phase, causing larger oil droplets to expand while smaller ones shrink [17]. In addition, after 100 days of storage at 40°C, the lemon myrtle nanoemulsion undergoes phase separation due to Brownian motion, leading to coalescence or flocculation and ultimately to the formation of larger droplets at high temperatures [37]. As noted earlier, Brownian motion can be accelerated at high temperatures. Accordingly, droplets may increase in size and travel in proximity to one another, thereby increasing the likelihood of instability.

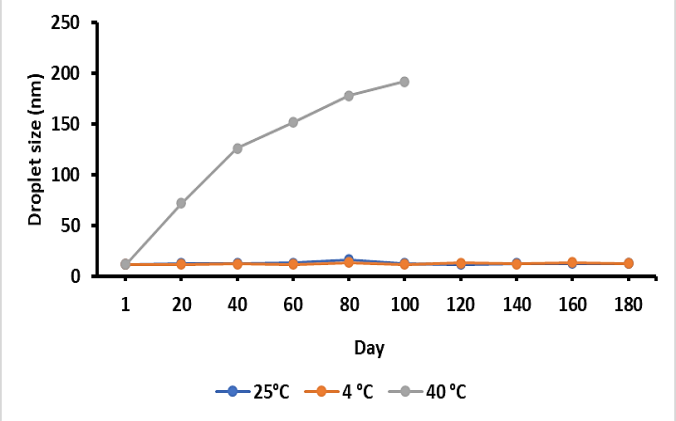

Figure 14. Effect of storage temperature (4°C, 25°C, and 40°C) on droplet size of optimized lemon myrtle EO nanoemulsion over 180 days. Results are expressed as mean ± SD (n = 3). Droplet size remained stable at 4°C and 25°C but increased significantly at 40°C, indicating reduced stability at higher storage temperatures.

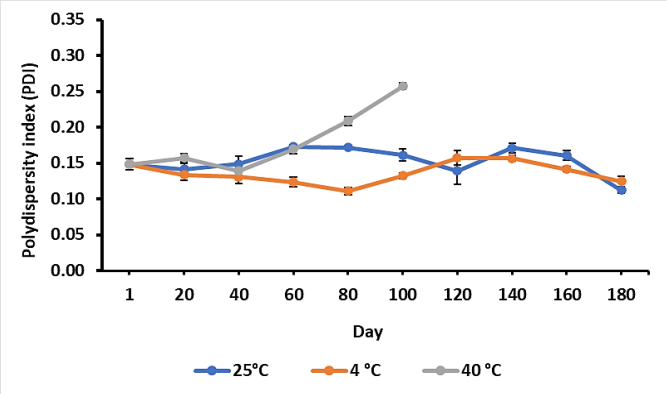

Figure 15. Effect of storage temperature (4°C, 25°C, and 40°C) on polydispersity index (PDI) of optimized lemon myrtle EO nanoemulsion over 180 days. Results are expressed as mean ± SD (n = 3). PDI remained below 0.173 at 4°C and 25°C, while a marked increase was observed at 40°C after 60 days, reflecting reduced uniformity.

Similarly, Nirmal et al. [14] found that after four weeks of storage at both 4 °C and 25 °C, the droplet size of their lemon myrtle oil nanoemulsion remained constant. Meanwhile, an increase in nanoemulsion droplet size was observed at a storage temperature of 40°C from 28.81 nm to 230.37 nm for LM-15 and from 16.07 nm to 80.52 nm for LM-17. Somala et al. [29] reported that *Cymbopogon nardus* EO nanoemulsion showed good stability at 4°C; however, the emulsion exhibited a larger droplet size, increasing from 79 nm to 218 nm after 14 days at 45 °C. Alderees et al. [1] also reported a similar finding: an increase in the size of Tasmanian pepper leaf EO nanoemulsion droplets was triggered by elevated storage temperatures. Meanwhile, the droplet size remained unchanged at a low storage temperature (5°C). Teng et al. [9] found that nanoemulsion stability is greatly affected by storage temperature and duration, and our results support their conclusions. In particular, the nanoemulsion oxidizes over time when left at room temperature, and the extent of oxidation increases with the storage time.

Any change in the nanoemulsion's interfacial composition, including oxidation of lipid components, is expected to weaken the emulsion's stability. As a result, the emulsifier may undergo a conformational change and eventually desorb from the interface. As a consequence of the breakdown of the nanoemulsion, the stored EO will be released, and phase separation will occur [29]. For nanodroplet compositions to maintain physical stability during storage, droplet size is a crucial factor to consider [1,7].

The ultrasonication method used in this study yielded a viable process for producing nanoemulsions containing lemon myrtle EO. The emulsion demonstrated excellent stability, withstanding storage at both 4°C and 25°C for up to 180 days. Our future work will focus on antibacterial and antioxidant evaluations of the optimized lemon myrtle EO nanoemulsion.

4. CONCLUSION

In this study, ultrasonication consistently produced a nanoemulsion formulation of lemon myrtle essential oil (EO). The optimum formulation parameters are as follows: 100% sonication amplitude for 5 minutes, with 1% (w/w) lemon myrtle EO and 4% (w/w) surfactant. This optimization resulted in a nanoemulsion with a mean droplet size of 12.52 nm and a PDI of 0.153, indicating a highly uniform, finely dispersed structure. In addition, the optimized nanoemulsion exhibited improved physical properties and extended stability. The optimized nanoemulsion also showed no alteration in the chemical constituents of the EO. To conclude, the findings supported the effectiveness of the ultrasonication approach in producing stable oil-in-water nanoemulsions. The findings highlight the strong potential of lemon myrtle essential oils as a delivery system with a wide range of applications across the food, agricultural, and pharmaceutical industries.

ACKNOWLEDGEMENT

The study was funded by the MARDI Development Fund (PRB-401).

REFERENCES

[1] F. Alderees, S. Akter, R. Mereddy, and Y. Sultanbawa, "Formulation, characterization, and stability of food-grade oil-in-water nanoemulsions of essential oils of *Tasmannia lanceolata*, *Backhousia citriodora* and *Syzygium anisatum*," *Journal of Food Safety*, vol. 42, no. 5, pp. 1–10, 2022.

[2] L. Pavoni, R. Pavela, M. Cespi, G. Bonacucina, F. Maggi and V. Zeni, "Green micro-and nanoemulsions for managing parasites, vectors and pests," *Nanomaterials*, vol. 9, no. 9, 2019.

[3] S. Sugumar, V. Ghosh, A. Mukherjee, and N. Chandrasekaran, "Essential oil-based nanoemulsion formation by low- and high-energy methods and their application in food preservation against food spoilage microorganisms", Essential Oils in Food Preservation, Flavor and Safety. *Elsevier Incor*., pp.93-100, 2016.

[4] I. F. Mustafa and M. Z. Hussein, "Synthesis and technology of nanoemulsion-based pesticide formulation," *Nanomaterials*, vol. 10, no. 8, pp. 1–26, 2020.

[5] M. Heydari, A. Amirjani, M. Bagheri, I. Sharifian, and Q. Sabahi, "Eco-friendly pesticide based on peppermint oil nanoemulsion: preparation, physicochemical properties, and its aphicidal activity against cotton aphid," *Environmental Science and Pollution Research*, vol. 27, no. 6, pp. 6667–6679, 2020.

[6] Y. Ozogul, E. Kuley Boga, I. Akyol, M. Durmus, Y. Ucar, and J.M. Regenstein, "Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens," *Food Bioscience*, vol. 36, 2020.

[7] I. R. Singh and A. K. Pulikkal, "Preparation, stability and biological activity of essential oil-based nano emulsions: A comprehensive review," *OpenNano*, vol. 8, no. June. Elsevier Inc., p. 100066, 2022.

[8] A.R. Mukurumbira, R.A. Shellie, R. Keast, E.A. Palombo, R. Shah, B.W. Muir, J. White, and S.R. Jadhav, "Preparation, physicochemical characterization and assessment of liquid and vapor phase antimicrobial activity of essential oil loaded lipid nanoparticles," *LWT*, Vol. 191, no.115624, 2024.

[9] F. Teng, M. He, F. Chen, C. Wu, Z. Wang, *et al.*, "Effect of ultrasonication on the stability and storage of a soy protein isolate-phosphatidylcholine nanoemulsion," *Scientific Reports,* vol. 10, no. 1, pp. 1–9, 2020.

[10] L. Salvia-Trujillo, M. A. Rojas-Graü, R. Soliva-Fortuny, and O. Martín-Belloso, "Impact of microfluidization or ultrasound processing on the antimicrobial activity against *Escherichia coli* of lemongrass oil-loaded nanoemulsions," *Food Control*, vol. 37, no. 1, pp. 292–297, 2014.

[11] A. Maurya, V.K. Singh, S. Das, J. Prasad, A. Kedia, N. Upadhyay, et al., "Essential oil nanoemulsion as eco-friendly and safe preservative: bioefficacy against microbial food deterioration and toxin secretion, mode of action, and future opportunities," *Frontiers in Microbiology*, vol. 12, pp. 1–17, 2021.

[12] L. Pavoni, D. R. Perinelli, G. Bonacucina, M. Cespi, and G. F. Palmieri, "An overview of micro-and nanoemulsions as vehicles for essential oils: Formulation, preparation and stability," *Nanomaterials*, vol. 10, no. 1, pp. 1–24, 2020.

[13] S. N. Bunawan, N. A. Masdor, N. A. Yasid, M. I. E. Halmi, and M. Y. Abd Shukor, "Effect of different hydrophilic-lipophilic balance (HLB) on droplet size, polydispersity index and stability of lemon myrtle nanoemulsion," *International Journal of Nanoelectronics and Materials,* vol. 16, no. December, pp. 431–440, 2023.

[14] N. P. Nirmal, R. Mereddy, L. Li, and Y. Sultanbawa, "Formulation, characterization and antibacterial activity of lemon myrtle and anise myrtle essential oil in water nanoemulsion," *Food Chemistry*, vol. 254, no. January, pp. 1–7, 2018.

[15] M. H. Shahavi, M. Hosseini, M. Jahanshahi, R. L. Meyer, and G. N. Darzi, "Evaluation of critical parameters for preparation of stable clove oil nanoemulsion," *Arabian Journal of Chemistry*, vol. 12, no. 8, pp. 3225–3230, 2019.

[16] S. Kumari, R.V. Kumaraswamy, R.C. Choudhary, S.S. Sharma, A. Pal, R. Raliya, et al., "Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean," *Scientific Reports*, vol. 8, no. 1, 2018.

[17] S. Moradi and A. Barati, "Essential oils nanoemulsions: Preparation, characterization and study of antibacterial activity against *Escherichia Coli*," *International Journal of Nanoscience and Nanotechnology*, vol. 15, no. 3, pp. 199–210, 2019.

[18] S. N. Liew, U. Utra, A. K. Alias, T. B. Tan, C. P. Tan, and N. S. Yussof, "Physical, morphological and antibacterial properties of lime essential oil nanoemulsions prepared via spontaneous emulsification method," *Lwt*, vol. 128, no. January, 2020.

[19] J. Nirmala, N. R, and R. Nagarajan, "Recent research trends in fabrication and applications of plant essential oil-based nanoemulsions, " Journal of Nanomedicine & Nanotechnology, vol. 08, no. 2, 2017.

[20] J. Carpenter and V. K. Saharan, "Ultrasonic assisted formation and stability of mustard oil in water nanoemulsion: Effect of process parameters and their optimization," *Ultrasonics Sonochemistry*, vol. 35, pp. 422–430, 2017.

[21] Y. Aisyah, S. Haryani, N. Safriani, and N. El Husna, "Optimization of emulsification process parameters of cinnamon oil nanoemulsion," *International Journal on Advanced Science Engineering and Information Technology*, vol. 8, no. 5, pp. 2092–2098, 2018.

[22] M. E. I. Badawy, A.-F. S. A. Saad, E.-S. H. M. Tayeb, S. A. Mohammed, and A. D. Abd-Elnabi, "Optimization and characterization of the formation of oil-in-water diazinon nanoemulsions: Modeling and influence of the oil phase, surfactant and sonication," *Journal of Environmental Science and Health,* Part B, vol. 52, no. 12, pp. 896–911, 2017.

[23] M. Liu, Y. Pan, M. Feng, W. Guo, X. Pan, J. Huang, and J. Cao, "Ultrasonics sonochemistry garlic essential oil in water nanoemulsion prepared by high-power ultrasound: Properties, stability and its antibacterial mechanism against MRSA isolated from pork," *Ultrasonics Sonochemistry*, vol. 90, no. August, p. 106201, 2022.

[24] G. P. Christy, D. A. Arimurni, M. D. P. Wahyudi, R. Martien, and W. A. S. Tunjung, "Formulation and characterization of kaffir lime oil nanoemulsion," *Biosciences, Biotechnology Research Asia*, vol. 14, no. 3, pp. 915–922, 2017.

[25] M. Firoozi, S. Rezapour-Jahani, Z. Shahvegharasl, and N. Anarjan, "Ginger essential oil nanoemulsions: Preparation and physicochemical characterization and antibacterial activities evaluation," *Journal of Food Process Engineering*, vol. 43, no. 8, 2020.

[26] N.A.N. Azmi, A.A.M. Elgharbawy, H.M. Salleh, and M. Moniruzzaman, "Preparation, characterization and biological activities of an oil-in-water nanoemulsion from fish by-products and lemon oil by ultrasonication method," Molecules, vol. 19, p. 6725, 2022.

[27] G. Polat Yemiş, E. Sezer, and H. Sıçramaz, "Inhibitory effect of sodium alginate nanoemulsion coating containing myrtle essential oil (*Myrtus communis* L.) on *Listeria monocytogenes* in Kasar Cheese," *Molecules*, vol. 27, no. 21, p. 7298, 2022.

[28] W. Tscharnuter, F. McNeil-Watson, and D. Fairhurst, "A new instrument for the measurement of very small electrophoretic mobilities using phase analysis light scattering," *ACS Symposium Series*, vol. 693, pp. 327–340, 1998.

[29] N. Somala, C. Laosinwattana, and M. Teerarak, "Formulation process, physical stability and herbicidal activities of *Cymbopogon nardus* essential oil-based nanoemulsion," *Scientific Reports*, vol. 12, no. 1, pp. 1–13, 2022.

[30] L. Salvia-Trujillo, A. Rojas-Graü, R. Soliva-Fortuny, and O. Martín-Belloso, "Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils," *Food Hydrocolloids*, vol. 43, pp. 547–556, 2015.

[31] S. Noori, F. Zeynali, and H. Almasi, "Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets," *Food Control*, vol. 84, pp. 312–320, 2018.

[32] S. Rao, P. Radhakrishnan, S. Valiathan, and S. M, "Rosehip oil nanoemulsion as a stable delivery system for omega-3 fatty acids to enhance the nutritional value of yogurt," *Food Chemistry Advances*, vol. 3, 2023.

[33] F. Chemat, I. Grondin, P. Costes, L. Moutoussamy, A. S. C. Sing, and J. Smadja, "High power ultrasound effects on lipid oxidation of refined sunflower oil," *Ultrasonics Sonochemistry*, vol. 11, no. 5, pp. 281–285, 2004.

[34] S. Agatonovic-Kustrin, P. Ristivojevic, V. Gegechkori, T. M. Litvinova, and D. W. Morton, "Essential oil quality and purity evaluation via FT-IR spectroscopy and pattern recognition techniques," *Applied Sciences (Switzerland)*, vol. 10, no. 20, pp. 1–12, 2020.

[35] Sultanbawa Y. "Lemon myrtle (Backhousia citriodora) oils", Essential Oils in Food Preservation, Flavor and Safety, *Academic Press*, pp. 517–521, 2016.

[36] V. Krstonošić, M. Milanović, and L. Dokić, "Application of different techniques in the determination of xanthan gum-SDS and xanthan gum-Tween 80 interaction," *Food Hydrocolloids*, vol. 87, pp. 108–118, 2019.

[37] C. M. Borba *et al.*, "Physical and chemical stability of β-carotene nanoemulsions during storage and thermal process," *Food Research International*, vol. 121, no. January, pp. 229–237, 2019.