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A Comparison of Sonication and Phase Inversion Temperature Methods for Formulating Lavender Essential Oil Nanoemulsions: Stability, Antioxidant Capacity, and Industrial Potential

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Abstract

Lavender essential oil (LEO) nanoemulsions are gaining attention for their enhanced stability and antioxidant properties, making them suitable for applications in pharmaceuticals, cosmetics, and the food industry. Despite the widespread use of the high-energy sonication method, the phase inversion temperature (PIT) method has been underexplored in the preparation of LEO-based nanoemulsions. This study aims to address this gap by comparing the efficacy of the low-energy PIT method with the high-energy sonication method in terms of stability and antioxidant capacity. The objectives were to evaluate the physical characteristics (particle size, polydispersity index (PDI), zeta potential, and turbidity) and the antioxidant capacity of nanoemulsions prepared by both methods. The results showed that PIT-prepared nanoemulsions, with slightly larger but more uniform droplet sizes (47.53 ± 0.306 nm, PDI: 0.408 ± 0.005), exhibited superior zeta potential (–14.60 ± 0.436 mV) and enhanced stability compared to those prepared by sonication (47.23 ± 0.252 nm, PDI: 0.466 ± 0.004). Furthermore, PIT-prepared nanoemulsions retained higher antioxidant capacity over a 30-day period. The techno-economic analysis revealed that while sonication is more energy-intensive, the PIT method offers better scalability and lower production costs. These findings suggest that the PIT method holds significant potential for large-scale industrial applications, offering a more cost-effective and sustainable approach for enhancing the stability and antioxidant capacity of LEO nanoemulsions.

Keywords: Lavender essential oil, Nanoemulsions, Phase inversion temperature, Sonication, Antioxidant capacity

Introduction

Lavender essential oil (LEO), derived from the native Mediterranean plant *Lavandula angustifolia Mill.*, is known for its aromatic qualities and its diverse biological capabilities. One of its primary applications is in aromatherapy, where it is known to alleviate stress, anxiety, and insomnia by inducing a calming and relaxing effect on the central nervous system [1,2]. Its antibacterial and antifungal effects have been explored in wound healing and infection prevention [3]. Furthermore, LEO has been investigated for its antioxidant activity, which plays a role in mitigating oxidative stress-related diseases and aging. Recent research also indicates potential uses in pain management, such as reducing muscle spasms and headaches, and in neurological disorders, like Alzheimer's disease, due to its neuroprotective properties [4,5]. LEO is rich in terpenoid and phenolic compounds, comprising over 150 different components. Among its primary constituents are the monoterpenoids linalool, linalyl acetate, 1,8-cineole, β-ocimene, terpinen-4-ol, and camphor [6]. However, LEO has several obstacles in food, cosmetics, perfumes, and

pharmaceutical applications, such as low solubility and low stability under external factors such as light, moisture, as well as temperature [7,8]. This contribution aims to encapsulate and synthesize lavender essential oil within the nanoemulsions system to address the challenges.

Nanoemulsions can be characterized as a colloidal dispersion comprising 2 immiscible liquids that are thermodynamically unstable when in contact, with droplet sizes typically ranging from 20 to 200 nm. Nanoemulsion encapsulation of natural bioactive compounds improves solubility and enables controlled release [9,10]. Nanoemulsion can be created via both high-energy and low-energy methods. Nanoemulsions can be created via both high-energy and low-energy methods, which differ in their approaches to droplet size reduction and energy consumption. High-energy techniques rely on mechanical devices to generate large disruptive forces, such as shear, turbulence, or cavitation, to break down droplets into nanosized particles. These methods, including microfluidization, high-pressure homogenization, and ultrasonication, have traditionally been the preferred choice due to their ability to produce highly uniform and stable nanoemulsions [11,12]. In contrast, low-energy techniques utilize changes in the physicochemical properties of the system, such as temperature, concentration, or phase behavior, to create nano-sized particles without the need for external mechanical forces. Methods like phase inversion temperature (PIT) and spontaneous emulsification fall under this category and have gained attention due to their mild processing conditions, making them suitable for temperature-sensitive components such as pharmaceutical or bioactive compounds [13]. While high-energy methods have been the standard due to their scalability and efficiency, the increasing demand for cost-effective and energy-efficient approaches has made low-energy techniques a more appealing choice, particularly for encapsulating delicate active ingredients in the pharmaceutical and food industries.

High-energy methods for preparing nanoemulsions employ mechanical devices such as microfluidizers, high-pressure homogenizers (HPH), and ultrasonicators, which generate nanodroplets through disruptive forces like shear, turbulence, and cavitation. These methods are industrially scalable and effective for reducing droplet sizes, even in highly viscous oils, while consuming fewer surfactants. However, they are energy-intensive, leading to higher production costs and potential challenges when working with heat-sensitive compounds [14–16]. Sonication is a widely utilized high-energy method for reducing the droplet size of nanoemulsions. This technique employs ultrasound waves (>20 kHz) to generate mechanical vibrations that create alternating high-pressure and low-pressure cycles within the emulsion system. These pressure variations induce microjets, shock waves, and particle collisions, resulting in the effective breakdown of droplets into nanosized particles [17].

Conversely, Low-energy methods for preparing nanoemulsions rely on the system's internal chemical energy or the chemical potential of its components to achieve emulsification. These methods induce changes in the surfactant's spontaneous curvature, enabling the formation of oil-in-water (o/w) or water-in-oil (w/o) nanoemulsions. Prominent techniques include spontaneous emulsification, phase inversion composition (PIC), and phase inversion temperature (PIT). Unlike high-energy methods, low-energy approaches are less energy-intensive, require mild processing conditions, and are well-suited for thermally sensitive bioactive compounds. Among these, the PIT method, which utilizes temperature-induced phase transitions, stands out for its high emulsification efficiency, low polydispersity index (PDI), and ability to produce stable nanoemulsions. These attributes make low-energy methods particularly appealing for pharmaceutical and food applications [18–22]. The PIT method not only offers technical advantages but also presents significant environmental and economic benefits compared to high-energy techniques. Its reliance on temperature shifts rather than mechanical force drastically reduces energy consumption, leading to lower operational costs and reduced carbon footprints [23,24]. Additionally, the mild processing conditions associated with the PIT method help preserve the integrity of heat-sensitive compounds, reducing waste and improving overall yield [25,26]. These attributes make it an attractive alternative for industries such as pharmaceuticals, cosmetics, and food, where cost-efficiency, sustainability, and product stability are critical [27,28]. Furthermore, its scalability and

reproducibility enhance its potential for widespread adoption in large-scale production processes [29].

Therefore, the aim of this study was to compare the nanoemulsion formation processes of lavender essential oil using 2 distinct methods, phase inversion temperature (PIT) and sonication. The focus was to enhance the stability and antioxidant capacity of the essential oil, making it suitable for potential applications in food, pharmaceutical, and cosmetic industries. We hypothesize that the PIT method will outperform sonication in terms of stability and antioxidant capacity, offering a more effective approach for improving the functional properties of lavender essential oil nanoemulsions.

Materials and methods

Materials

Lavender essential oil (LEO) was obtained by distillation of seeds and was supplied from the Organic International Joint Stock Company, in Ho Chi Minh City, Vietnam. Tween 80 (polyethylene glycol sorbitan monooleate) as surfactant was purchased from Xilong Scientific Co., Ltd., Guangdong, China. DPPH (2,2-diphenyl-1-picrylhydrazyl) and Ascorbic acid were purchased from Sigma-Aldrich, Singapore. Double-distilled water was used throughout the study, prepared in the Laboratory of Biofuel and Biomass Research, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology, Vietnam. All other chemicals were analytical grade and were used without further purification.

Gas chromatography-mass spectrometry (GC-MS) analysis of lavender essential oil

GC-MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph (Les Ulis, France), paired with an Agilent Technologies 5973 quadrupole mass spectrometer (Les Ulis, France) and supported by a Gerstel MPS autosampler (Müllheim an der Ruhr, Germany). The separation of chemical compounds was performed on an HP5-MS column with helium as the carrier gas at a steady flow rate of 1 mL/min. The injection port temperature was set at 250 °C, with the helium head pressure regulated at 9.3 psi. Samples were diluted in hexane prior to analysis. Identification of the compounds was based on a comparison of retention indices with data from the Wiley Library and corroborated using reference information from existing mass spectrometry literature.

Nanoemulsions preparation

Sonication method

The LEO nanoemulsion was prepared by mixing 10 % (v/v) LEO, 16 % (v/v) Tween 80, 20 % (v/v) ethanol, and 54 % distilled water. The mixture was initially stirred using a magnetic stirrer at 1,000 rpm for 30 min to create a coarse emulsion. Following this, the coarse emulsion was subjected to sonication using an ultrasonic processor, operating at 750 W power, 20 kHz frequency, and a pulse duration of 1 sec ON/2 sec OFF for 15 min. The temperature of the emulsion was monitored during sonication and was kept at 25 ± 2 °C using an ice bath to prevent overheating. The resulting nanoemulsion exhibited no phase separation and demonstrated excellent transparency, indicating successful formulation [30,31].

PIT method

The nanoemulsion was prepared using the same formulation as the Sonication method, but via the phase inversion temperature (PIT) method, with modifications based on the procedure described by Rao *et al.* [21], with some modifications. In brief, the oil phase mixture was added to the heated water phase at 60 ± 2 °C, and the mixture was continuously stirred until the PIT temperature of approximately 90 ± 2 °C was reached. The temperature was carefully monitored using a digital thermometer during the heating phase to ensure a gradual and controlled temperature rise. The emulsion was then stirred for an additional 45 min to develop a coarse emulsion. Following this, the system was rapidly cooled by immersion in an ice bath (4 ± 2 °C), resulting in the formation of stable nanoemulsions. The final emulsion displayed an oil-in-water composition, with no phase separation observed.

Characterizations

Particle size, polydispersity index (PDI), and zeta potential measurement

The particle size, polydispersity index (PDI), and ζ-potential of the nanoemulsion were measured using dynamic light scattering (DLS) with a Zetasizer Nano ZS 90 Nanoparticle Size Analyzer (Malvern Instruments, Worcestershire, UK) [32].

Transmission electron microscopy (TEM) measurement

The surface morphology of the nanoemulsion droplets was analyzed using Transmission Electron Microscopy (TEM) with a JEOL JEM-1400 Plus microscope (Akishima, Tokyo, Japan). Briefly, 50 µL of the sample was adsorbed onto 200-mesh formvar-coated copper sample holders for 1 min. The droplets were then negatively stained with 50 µL of 1.5 % (w/v) phosphotungstic acid for 10 min at room temperature. After removing the excess stain, the sample holders were examined using TEM, which was operated at 67 kV with a 20 µm aperture [30].

Turbidity measurement

The turbidity of the nanoemulsions was assessed by measuring their absorbance at 660 nm using a UV-Vis spectrophotometer without prior dilution. Distilled water served as the blank control. The turbidity was calculated using the following equation [33]:

$$T = 2.303A/L$$

where T = turbidity of nanoemulsions (cm^{-1}), A = absorbance value at 660 nm and L = path length of the cuvette (cm).

Nanoemulsion stability

The prepared nanoemulsions were stored at room temperature (25 ± 2 °C), in the refrigerator (4 ± 2 °C) for 30 days, a further analysis was performed to assess alterations in droplet size and PDI values, following the procedure described earlier.

Antioxidant capacity

The antioxidant capacity of the nanoemulsions was used to measure the scavenging of free radicals of DPPH as described by Nie *et al.* [35], with some modifications. In brief, 0.15 mL of the nanoemulsion sample was mixed with 2.85 mL of 0.1 mM DPPH solution. The mixture was vortexed thoroughly and incubated in the dark at room temperature for 30 min. The absorbance of the samples was then measured at 517 nm using a UV-Vis spectrophotometer (Cary 60 UV–Vis, Agilent Technologies, USA).

A calibration curve was prepared using ascorbic acid (AA) as the standard. Specifically, 0.15 mL of AA solutions at concentrations of 50, 100, 150, 200, 400, and 600 µM were mixed with 2.85 mL of DPPH solution. The mixtures were vortexed, incubated in the dark at room temperature for 30 min, and their absorbance values were measured under the same conditions.

The antioxidant capacity of the nanoemulsions was expressed as micrograms of ascorbic acid equivalent per gram of bioactive compound (µg AA/g).

Statistical analysis

The experimental procedures were repeated 3 times and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA and 2-way ANOVA followed by Tukey's test, utilizing the SPSS Statistics 26.0 software. A significance level of $p < 0.05$ was used to determine the notable differences between samples.

Before performing ANOVA, assumptions regarding normality and homogeneity of variance were tested to ensure the validity of the statistical tests. The normality of the data was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. If any of these assumptions were not met, appropriate data transformations were applied, or non-parametric methods were considered. All statistical analyses were performed at a significance level of $p < 0.05$.

Results and discussion

Identification of the components of lavender essential oil

The analysis of lavender essential oil reveals a diverse range of chemical constituents, where **Table 1** details the chemical composition, and **Figure 1** represents the corresponding gas chromatography spectrum of lavender essential oil. The major composition is dominated by linalyl acetate (38.47 %) and linalool (23.67 %), which collectively account for over 60 % of the total content. These compounds are well-documented for their significant roles in imparting the characteristic aroma and biological activities of lavender essential oil, including antimicrobial, anti-inflammatory, and anxiolytic effects [2,3]. Additionally, secondary components such as D-limonene (8.74 %)

and eucalyptol (7.62 %) provide antioxidant benefits and support respiratory health [34]. Notable contributions are also made by β-pinene (6.21 %) and α-pinene (4.87 %), which exhibit antimicrobial and anti-inflammatory properties, further enhancing the therapeutic value of the oil [35]. Several minor components, including nerol (0.96 %), camphor (0.65 %), and β-citronellol (0.79 %), contribute to the complexity of the oil. These constituents, while present in lower concentrations, may have synergistic effects with the major compounds, enhancing the overall biological activity of the essential oil [36]

Table 1 Chemical composition of lavender essential oil.

No.	Retention time	Constituent	Content (%)
1	7.633	α-Pinene	4.87
2	8.355	Camphene	0.08
3	9.752	β-Pinene	6.21
4	10.097	1-Octen-3-ol	0.09
5	10.583	β-Myrcene	2.00
6	12.470	o-Cymene	0.23
7	12.769	D-Limonene	8.74
8	12.875	Eucalyptol	7.62
9	13.367	cis-Ocimene	0.18
10	14.005	trans-Ocimene	0.26
11	14.593	γ-Terpinene	0.10
12	17.560	Linalool	23.67
13	19.796	1,2-Dihydrolinalool	0.24
14	20.099	Camphor	0.65
15	21.598	Borneol	0.34
16	22.098	4-Terpineol	0.30
17	22.857	α-Terpineol	0.09
18	24.300	Nerol	0.96
19	24.476	β-Citronellol	0.79
20	24.604	Isopulegol acetate	0.22
21	25.459	Linalyl acetate	38.47
22	26.456	β-Terpinyl acetate	0.21
23	26.585	γ -Terpinyl acetate	0.18
24	26.714	N/A	0.22
25	28.780	α-Terpineol acetate	2.08
26	28.840	N/A	0.54
27	29.222	Nerylacetate	0.15
28	29.840	Geranyl acetate	0.27
29	31.017	trans-Caryophyllene	0.18
30	32.061	trans-β-Famesene	0.08

N/A: Not assesssed

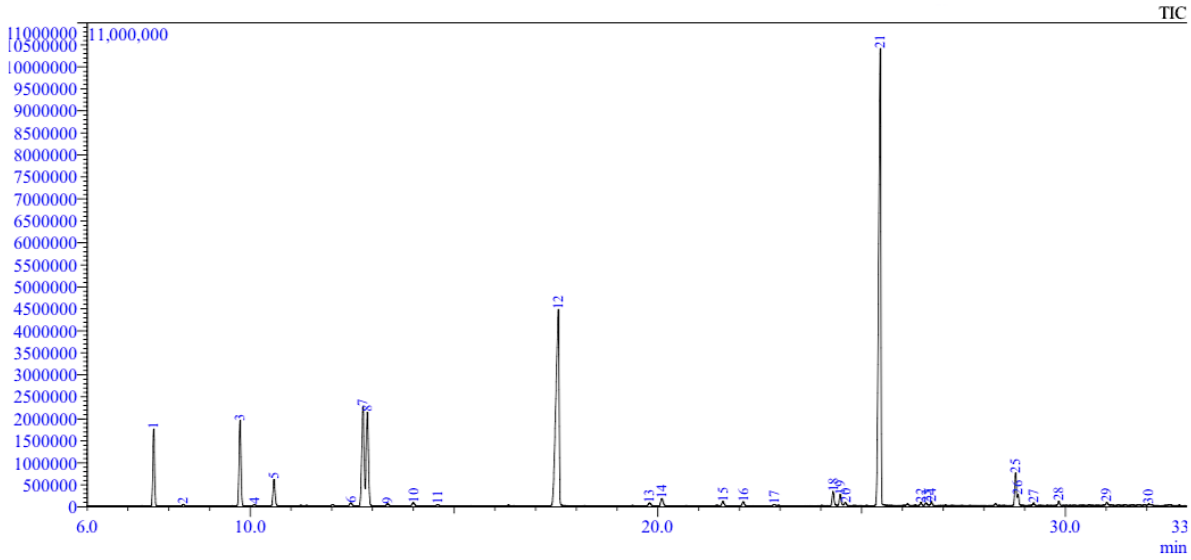

Figure 1 Gas chromatography spectrum of lavender essential oil.

Nanoemulsion characterizations

The characterization of nanoemulsions prepared via PIT and sonication methods provides critical insights into the impact of fabrication techniques on their physicochemical properties. Parameters such as particle size, polydispersity index (PDI), zeta potential, and turbidity reveal the fundamental differences between these 2 preparation methods and their implications for nanoemulsion stability and functionality (**Table 2**).

The nanoemulsions prepared using the sonication and PIT method demonstrated comparable particle sizes, with the sonication method yielding a particle size of 47.23 ± 0.252 nm and a polydispersity index (PDI) of 0.466 ± 0.004, while the PIT method resulted in particles measuring 47.53 ± 0.306 nm with a PDI of 0.408 ± 0.005 (**Table 2**). Although the size difference is minimal, the marginally smaller droplets in the sonicated samples suggest that the mechanical forces generated during sonication effectively disrupt and reduce droplet size. The nano-scale droplet sizes observed in both methods are beneficial for stability, as they minimize gravitational separation, such as creaming or sedimentation, and promote homogeneity within the system. The PDI value serves as a measure of the particle size distribution within the emulsion particles, indicating the uniformity of droplet size. A PDI value lower than or equal to 0.30 suggests a high level of uniformity and a narrower size distribution among the particles [37,38]. As indicated in **Table 2**, nanoemulsions prepared using PIT showed a PDI of 0.408 ± 0.005, while those produced by sonication exhibited a slightly higher PDI of 0.466 ± 0.004. Both values are below the acceptable threshold of 0.5 for monodispersity, indicating relatively homogeneous droplet distributions. However, the higher PDI in sonicated samples suggests a broader size distribution, potentially arising from the mechanical fragmentation process inherent in sonication. By contrast, the PIT method, which relies on thermal energy for phase inversion, promotes the spontaneous formation of nano-scale droplets with more uniform sizes. Despite these differences, both methods achieved PDIs indicative of stable nanoemulsions, suitable for practical applications.

Additionally, **Figure 2** represents the size distributions by intensity of the prepared nanoemulsions by sonication and PIT methods. When the sonication method was applied, the system exhibited 2 distinct particle size ranges, suggesting the presence of droplets with varying sizes. This could be attributed to the high-energy ultrasonic waves causing uneven fragmentation of droplets, leading to a broader size distribution. In contrast, the nanoemulsion prepared using the PIT method demonstrated a significantly narrower particle size distribution, characterized by a single, uniform peak. This indicates a more homogeneous droplet size distribution, likely due to the controlled phase inversion process. The PIT method facilitates the formation of droplets with uniform sizes by creating a structured

interfacial layer at the inversion temperature, ensuring greater stability and consistency in the emulsion system.

Table 2 Characterization of nanoemulsion preparation. The data presented are the mean ± standard deviation (n = 3).

	Particle size (nm)	PDI	Zeta potential (mV)	Turbidity (OD at 660 nm)
PIT	47.53 ± 0.306	0.408 ± 0.005	–14.60 ± 0.436	0.016 ± 0.004
Sonication	47.23 ± 0.252	0.466 ± 0.004	–12.30 ± 0.306	0.014 ± 0.002

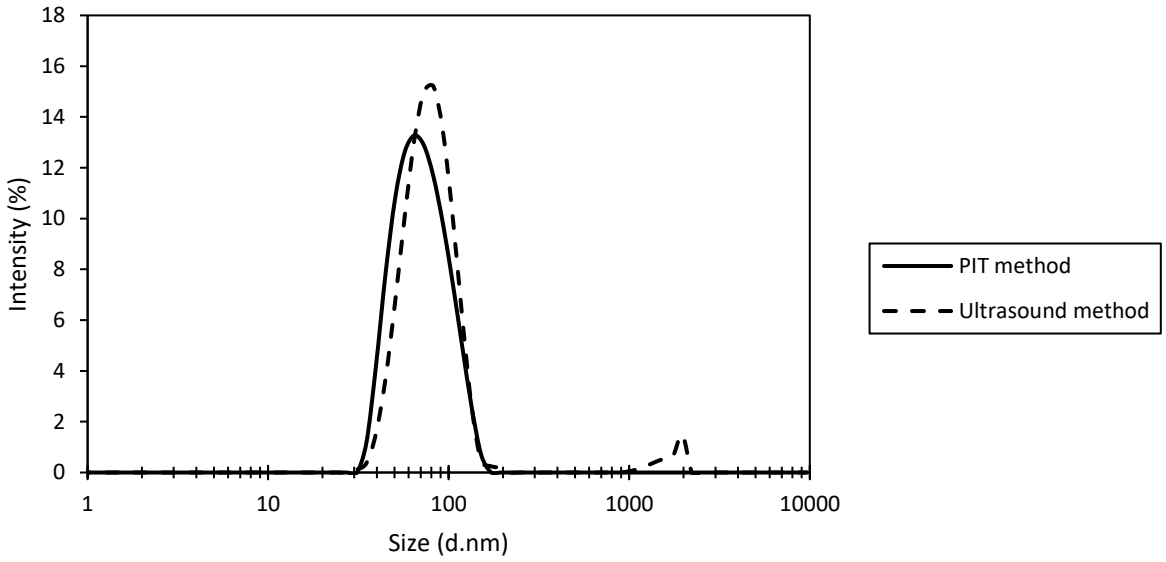

Figure 2 Particle size distributions by intensity of nanoemulsions were obtained by applying sonication and PIT methods.

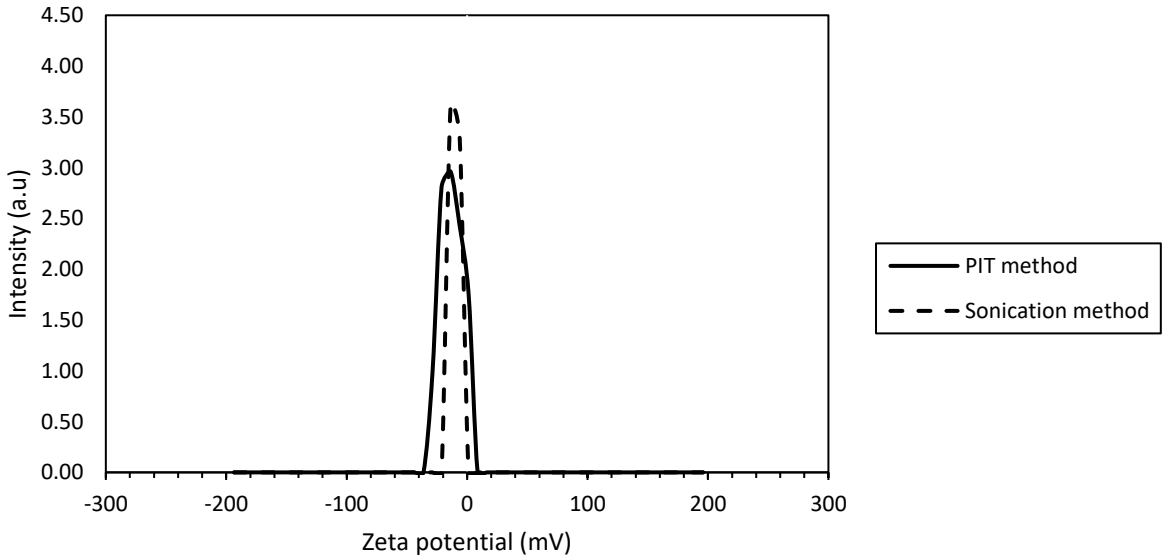

Figure 3 The zeta potential value of the nanoemulsion prepared via sonication and PIT methods.

Measurement of the zeta potential value offers valuable insights into the electrical charge carried by nanoemulsion droplets, which serves as a key important for the evaluation of the stability of the colloidal suspension stability [39]. In our study, it can be observed in nanoemulsions produced by the sonication method (–

12.30 ± 0.306 mV), while the opposite trend was observed for those synthesized using the PIT method (–14.60 ± 0.436 mV). Typically, nanoemulsions with zeta potential values below the absolute 30 mV tend to exhibit poor stability, manifesting in tendencies for agglomeration and coalescence [39]. In contrast, nanoemulsions that exceed the 30-mV threshold are considered stable, primarily due to electrostatic repulsion forces [40]. Thus, our findings suggest that despite the negative value of the zeta potential, EO-based nanoemulsions are considered less stable in both methods.

In addition, the turbidity of nanoemulsions prepared using the sonication and PIT methods was assessed through absorbance measurements. The results indicated that the nanoemulsion produced by the PIT method exhibited an absorbance value of 0.016 ± 0.004, while the sonication method produced a slightly lower absorbance of 0.014 ± 0.002. These findings suggest that the nanoemulsion synthesized through the PIT method tends to have slightly higher turbidity than the one prepared by using sonication. The differences in turbidity may be attributed to variations in droplet size distribution, interfacial tension, or degree of adsorption of the emulsifier between the two preparation methods.

The TEM analysis of nanoemulsions prepared by both the phase inversion temperature (PIT) method and the sonication method revealed well-defined spherical droplets with particle sizes measuring approximately 45 nm and 40 nm, respectively, as shown in **Figure 4**. The uniformity of the spherical droplet morphology observed in the TEM images aligns closely with the particle size data obtained from dynamic light scattering (DLS), confirming the consistency and reliability of the droplet size measurements across both methods. Notably, while both methods successfully produced nanoemulsions with similar droplet sizes and morphology, the intensity and uniformity of the droplets appeared more pronounced in the nanoemulsions prepared via the PIT method compared to those prepared by sonication. This difference may be attributed to the extended mixing and rapid cooling process in the PIT method, which could facilitate a more stable emulsification process and the formation of droplets with higher uniformity. Conversely, the sonication method, while effective in reducing particle size rapidly, may result in slightly less intense droplet formation due to variations in the ultrasonic energy distribution during the emulsification process.

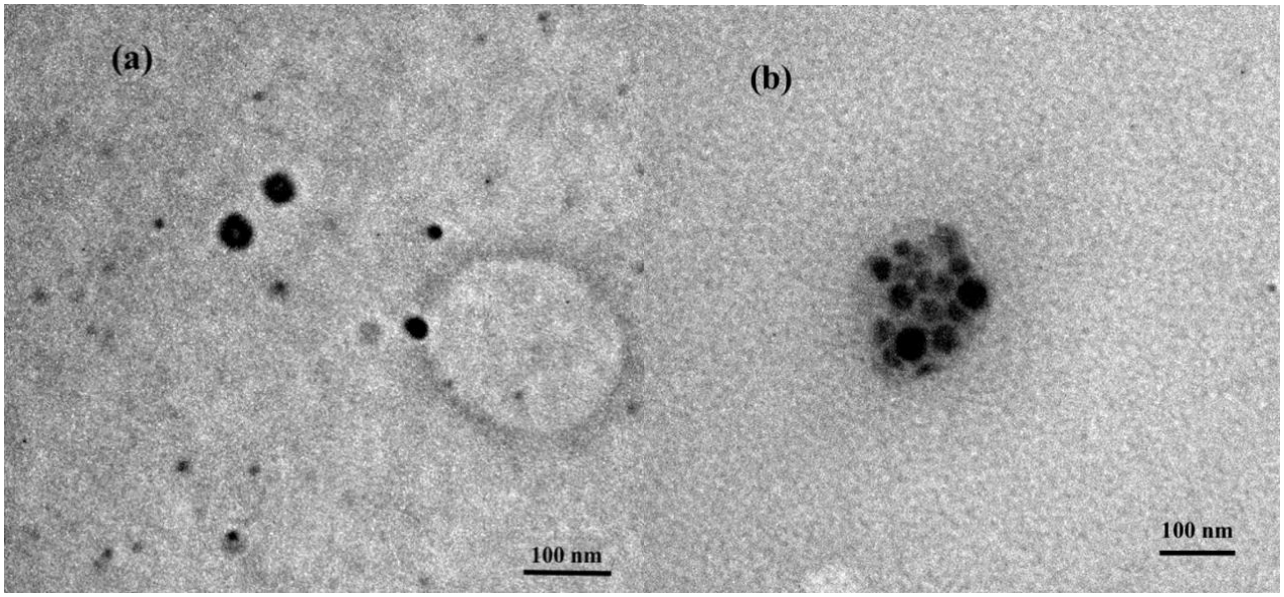

Figure 4 TEM images of LEO-based nanoemulsions formulated (a) PIT and (b) sonication methods at 100 nm scale bars.

Effects of storage conditions and storage times on nanoemulsion stability

The influence of storage duration and temperature on the stability of the nanoemulsions was investigated and characterization was evaluated according to particle size, PDI, and turbidity measurements.

Particle size is a fundamental parameter influencing the physicochemical stability of

nanoemulsions, as changes in size can indicate destabilization processes such as coalescence or Ostwald ripening. The results presented in **Figure 5(a)** reveal that initial particle size distributions were comparable across both storage conditions, suggesting that the preparation methods (sonication and PIT) effectively produced uniform nanoemulsions at day 0. Following the 30-day observation period, a statistically significant difference ($p < 0.05$) was observed in nanoemulsions across varying storage durations and temperatures between the two methods, sonication, and PIT methods. In samples stored at room temperature, particle size exhibited a noticeable increase from day 10 to day 20, followed by a slight decrease by day 30. This transient increase in size could be attributed to coalescence, where droplets merge due to thermal motion, followed by potential phase separation or precipitation as larger droplets sediment. In contrast, nanoemulsions stored under refrigeration showed minimal changes in particle size over the storage period, indicating that lower temperatures effectively slowed down destabilizing processes.

As shown in **Figure 5(b)**, PDI values were initially identical in both storage environments at day 0, reflecting consistent starting conditions across samples. However, as storage progressed, room temperature samples displayed a gradual increase in PDI, with a statistically significant difference ($p < 0.05$) observed by day 30 compared to refrigerated samples. This increase in PDI at room temperature indicates a trend towards greater size heterogeneity, likely driven by destabilization phenomena such as flocculation, coalescence, and droplet aggregation. Elevated temperatures at room conditions enhance molecular motion, which may accelerate emulsifier desorption or structural rearrangements at the oil-water interface, resulting in a loss of droplet stability. Additionally, environmental factors such as light exposure and temperature fluctuations can exacerbate these destabilization mechanisms. In contrast, refrigerated samples demonstrated relatively stable PDI values over the storage period, with only minor fluctuations. The cooler temperature likely slowed destabilization processes, preserving the uniformity of droplet size distribution.

Turbidity also serves as a visual and quantitative indicator of nanoemulsion stability, as increased turbidity often signifies structural changes such as droplet aggregation, phase separation, or precipitation. As shown in **Figure 5(c)**, turbidity levels were consistent across all samples at day 0, reflecting high initial clarity and structural uniformity. Over the 30-day storage period, distinct trends emerged between the two storage conditions ($p < 0.05$). Samples stored at room temperature exhibited a progressive increase in turbidity, particularly from day 10 onwards, suggesting a decline in optical clarity due to the destabilization of the nanoemulsion system. This trend aligns with the observed increase in particle size and PDI under the same conditions, reinforcing the hypothesis that destabilization processes, such as aggregation and phase separation, are more pronounced at elevated temperatures. In contrast, refrigerated samples displayed relatively minor changes in turbidity, indicating better preservation of optical clarity and structural integrity. The cooler environment likely suppressed destabilization mechanisms, thereby maintaining the transparency and quality of the nanoemulsions over time.

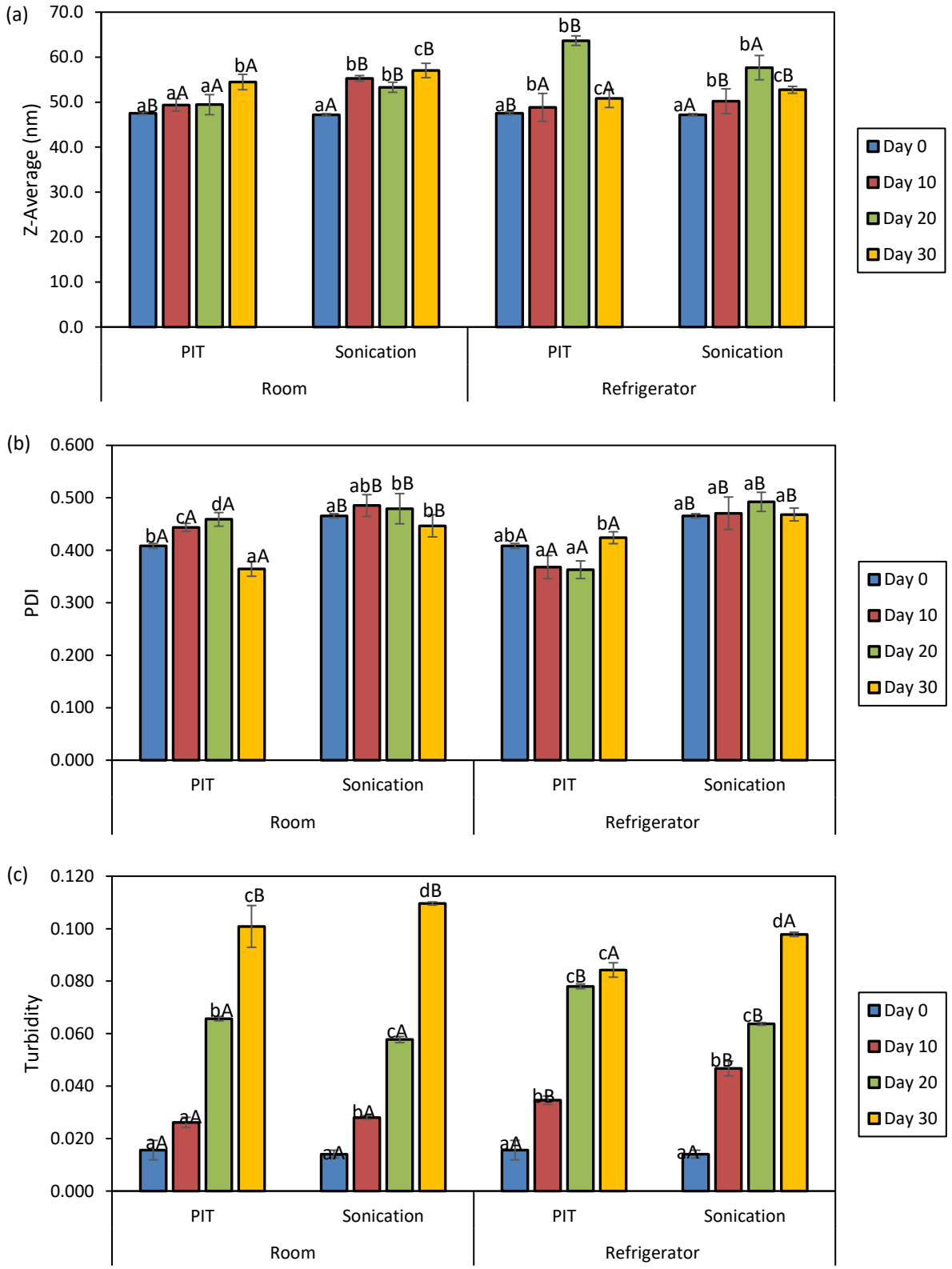

Figure 5 The (a) particle size, (b) PDI, and (c) turbidity of the nanoemulsion stored at room temperature and in the refrigerator for 30 days. Values are means ± standard deviation. The error bars represent the standard deviation. The same lowercase letters are significantly different ($p < 0.05$) by Tukey's test for storage times. The same uppercase letters are significantly different ($p < 0.05$) in Tukey's test for storage conditions.

Antioxidant capacity

Figure 6 reports a comparative analysis of the antioxidant capacity of coarse emulsion and nanoemulsions prepared using the Sonication and PIT methods. The results reveal a significant difference in the antioxidant capacities among these formulations ($p < 0.05$). Over the 30-day period, nanoemulsions prepared by the PIT method exhibited the highest retention of antioxidant capacity, followed by the Sonication method, with the coarse emulsion significantly underperforming.

On Day 0, the PIT method produced nanoemulsions with the highest antioxidant capacity (1089.90 μg AA/g), surpassing the Sonication method (1011.25 μg AA/g) and far exceeding the coarse emulsion (99.32 μg AA/g). The superior performance of the PIT method can be attributed to its ability to create highly uniform and smaller droplet sizes due to precise temperature control during emulsification. Smaller droplets provide a greater interfacial surface area, allowing for efficient encapsulation and protection of antioxidants from degradation, as supported by prior studies demonstrating the effectiveness of droplet size uniformity in enhancing oxidative stability [41]. In contrast, the coarse emulsion's larger droplets exhibited minimal encapsulation efficiency, exposing a greater proportion of antioxidants to oxidative degradation and environmental stress.

During storage, the antioxidant capacity of all emulsions declined; however, the rate and extent of degradation varied significantly across the formulations. After 30 days, the PIT nanoemulsion retained 68.7 % of its initial antioxidant capacity (749.24 μg AA/g), compared to 61.2 % retention for the Sonication nanoemulsion (618.55 μg AA/g). The PIT method's ability to stabilize antioxidants over time is likely due to the thermodynamic stability and smaller droplet size distribution achieved during preparation, as reported by Sharif *et al.* [42]. This enhanced stability minimizes the diffusion of oxidative agents such as oxygen into the emulsion droplets, thus slowing the degradation process.

Meanwhile, the coarse emulsion retained only 24.9 % of its initial antioxidant capacity (24.77 μg AA/g) after 30 days. This substantial loss underscores the limitations of conventional emulsification techniques, where larger droplet sizes and uneven distribution leave antioxidants more exposed to environmental factors such as light and oxygen. Previous studies have similarly shown that emulsions with larger droplet sizes are prone to coalescence and oxidation, leading to rapid degradation of bioactive compounds [41].

Although both nanoemulsification techniques significantly outperformed the coarse emulsion, the PIT method consistently exhibited superior antioxidant retention. The Sonication method, while effective in producing smaller droplets through acoustic cavitation, may inadvertently generate localized heat and free radicals during emulsification, potentially compromising antioxidant stability. This aligns with findings by Leong *et al.* [43], which noted that mechanical forces in ultrasonic emulsification can lead to partial degradation of sensitive bioactive. In contrast, the PIT method avoids these issues by leveraging temperature-induced phase inversion to achieve a finer and more stable droplet size distribution. The superior antioxidant retention observed in the PIT method can be attributed to several key factors, including the formation of a protective interfacial layer around the droplets. During phase inversion, the composition of surfactants and oils in the system undergoes a transition that enhances the stability of the nanoemulsion. This phase inversion creates a more compact and stable interfacial structure that effectively shields the bioactive compounds, such as antioxidants, from degradation. The transition from a water-in-oil to an oil-in-water system, which occurs at the phase inversion point, results in droplets with smaller sizes and a more uniform distribution. These smaller droplets increase the surface area for antioxidant encapsulation, thereby enhancing their retention [44,45]

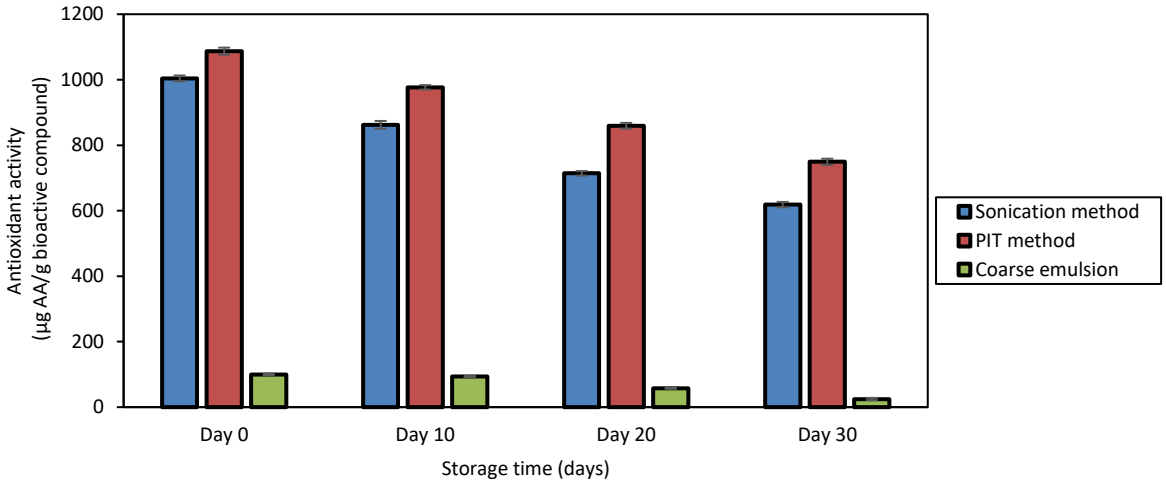

Figure 6 Antioxidant capacity of nanoemulsions prepared by sonication and PIT methods compared to coarse emulsions over 30 days. Values are means ± standard deviation. The error bars represent the standard deviation.
Coarse emulsion: LEO enriched with Tween 80 and Ethanol.
Sonication method: nanoemulsion of 10 % LEO, 16% Tween 80, 20 % Ethanol and water prepared by sonication method.
PIT method: nanoemulsion of 10 % LEO, 16 % Tween 80, 20 % Ethanol and water prepared by PIT method.
AA: ascorbic acid.

Techno-economic analysis and scalability challenges

The techno-economic analysis of nanoemulsion production highlights several key factors influencing overall costs, including raw material prices, energy consumption, and processing equipment costs [46]. Among the various production techniques, sonication is one of the most used methods. However, scalability challenges related to energy requirements and the cost of specialized equipment need to be addressed to make this method viable for large-scale applications.

Table 3 reports a summary of the estimated costs for both sonication and PIT methods. The sonication method, though widely used, exhibits higher costs due to its energy-intensive nature and the specialized equipment required. Interestingly, the total production cost for sonication is approximately 29.00 USD/kg, nearly 2.5 times higher than the 12.00 USD/kg required for the PIT method. Furthermore, the initial equipment cost for the sonication method ranges from 1,500 to 3,000 USD, significantly exceeding the PIT method, which typically requires an investment of 500 to 1,000 USD. These cost differences can be primarily attributed to the higher energy consumption and equipment expenses associated with sonication [47,48]

Table 3 Summary of production costs for nanoemulsion preparation methods.

Process Step	Sonication method (USD/kg)	PIT method (USD/kg)
Energy consumption	8.50	4.50
Raw materials	8.00	8.00
Labor costs	6.00	6.00
Maintenance costs	6.50	3.50
Total	29.00	12.00

Scalability challenges are also significant, especially for the sonication method, which is limited by the high energy demand and the cost of specialized equipment. These challenges need to be addressed to make the sonication method viable for large-scale applications [48]. The PIT method, in contrast, offers

better scalability, relying on temperature variations rather than mechanical input. However, it still requires precise control over heating and cooling cycles, which can lead to additional costs when scaled up [49]. According to Fernanda Brito de Carvalho-Guimarães and co-workers, producing stable emulsions on an industrial scale requires overcoming technical challenges related to process efficiency and energy consumption [50]. Furthermore, Safaya *et al.* [16] emphasized the importance of overcoming scalability issues when transitioning from lab-scale to industrial production, particularly in maintaining emulsion stability and controlling costs.

Conclusions

In this study, we have demonstrated that both the phase inversion temperature (PIT) and sonication methods are effective in producing stable and bioactive lavender essential oil (LEO) nanoemulsions with droplet sizes of < 50 nm. These nanoemulsions exhibit enhanced stability and antioxidant capacity, making them suitable for various applications in the pharmaceutical, cosmetic, and food industries. Among the 2 methods, the PIT method produced nanoemulsions with a more uniform droplet size distribution (lower PDI) and superior electrostatic stability, as indicated by more negative zeta potential values. These features contribute to the PIT method's enhanced long-term stability compared to the sonication approach. In terms of antioxidant capacity, both methods successfully preserved the bioactivity of LEO. However, the PIT-prepared nanoemulsions demonstrated slightly higher antioxidant retention over 30 days, making them particularly beneficial for applications requiring sustained bioactivity.

Furthermore, the sonication method also produced effective emulsions, but it is more energy-intensive and less scalable, which could limit its suitability for large-scale production. In contrast, the PIT method offers greater scalability and cost-effectiveness, making it a promising solution for industrial applications, as demonstrated by the techno-economic analysis. These advantages position the PIT method as a more sustainable option for large-scale emulsion production. Future research should focus on further optimizing the PIT process to enhance nanoemulsion stability, uniformity, and bioactivity, as well as addressing challenges related to scalability and cost-effectiveness. Combining PIT with advanced techniques, such as microfluidization or high-pressure homogenization, could further improve nanoemulsion performance. Expanding the use of PIT to other bioactive compounds presents exciting opportunities for developing efficient and sustainable emulsion technologies across diverse industries, particularly those requiring large-scale production.

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References

[1] FV Romeo, SD Luca, A Piscopo and M Poiana. Antimicrobial effect of some essential oils. *Journal of Essential Oil Research* 2008; **20(4)**, 373-379.

[2] HMA Cavanagh and JM Wilkinson. Biological activities of lavender essential oil. *Phytotherapy Research* 2002; **16(4)**, 301-308.

[3] AT Peana, PS D'Aquila, F Panin, G Serra, P Pippia and MDL Moretti. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine* 2002; **9(8)**, 721-726.

[4] M Lis-Balchin and S Hart. Studies on the mode of action of the essential oil of lavender (lavandula angustifolia P. miller). *Phytotherapy Research* 1999; **13(6)**, 540-542.

[5] PH Koulivand, MK Ghadiri and A Gorji. Lavender and the nervous system. *Evidence-Based Complementary and Alternative Medicine* 2013; **2013**, 681304.

[6] S Kasper, M Gastpar, WE Muller, H Volz, H Moller, A Dienel and S Schlafke. Efficacy and safety of silexan, a new, orally administered lavender oil preparation, in subthreshold anxiety disorder - evidence from clinical trials. *Wiener Medizinische Wochenschrift* 2010; **160(21-22)**, 547-556.

[7] H Vaskova and M Buckova. Thermal degradation of vegetable oils: Spectroscopic measurement and analysis. *Procedia Engineering* 2015; **100**, 630-635.

[8] C Turek and FC Stintzing. Stability of essential oils: A review. *Comprehensive Reviews in Food Science and Food Safety* 2013; **12(1)**, 40-53.

[9] C Qian and DJ McClements. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids* 2011; **25(5)**, 1000-1008.

[10] M Goutayer, S Dufort, V Josserand, A Royere, E Heinrich, F Vinet, J Bibette, J Coll and I Texier. Tumor targeting of functionalized lipid nanoparticles: Assessment by *in vivo* fluorescence imaging. *European Journal of Pharmaceutics and Biopharmaceutics* 2010; **75(2)**, 137-147.

[11] A Jintapattanakit. Preparation of nanoemulsions by phase inversion temperature (PIT). *Pharmaceutical Sciences Asia* 2018; **42(1)**, 1-12.

[12] H Cinar and H Alkan. Crustal s-wave structure around the lake van region (Eastern Turkey) from interstation rayleigh wave phase velocity analyses. *Turkish Journal of Earth Sciences* 2017; **26(1)**, 73-90.

[13] M Koroleva and EV Yurtov. Nanoemulsions: The properties, methods of preparation and promising applications. *Russian Chemical Reviews* 2012; **81(1)**, 21-43.

[14] N Anton, J Benoit and P Saulnier. Design and production of nanoparticles formulated from nano-emulsion templates - a review. *Journal of Controlled Release* 2008; **128(3)**, 185-199.

[15] DJ McClements. Edible nanoemulsions: Fabrication, properties, and functional performance. *Soft Matter* 2011; **7(6)**, 2297-2316.

[16] M Safaya and YC Rotliwala. Nanoemulsions: A review on low energy formulation methods, characterization, applications and optimization technique. *Materials Today Proceedings* 2020; **27(Part 1)**, 454-459.

[17] A Pratap-Singh, Y Guo, SL Ochoa, F Fathordoobady and Singh A. Optimal ultrasonication process time remains constant for a specific nanoemulsion size reduction system. *Scientific Reports* 2021; **11(1)**, 9241.

[18] G Ren, Z Sun, Z Wang, X Zheng, Z Xu, D Sun. Nanoemulsion Formation by the Phase Inversion Temperature Method Using Polyoxypropylene Surfactants. *Journal of Colloid and Interface Science* 2019; **540**, 177-184.

[19] G Lefebvre, J Riou, G Bastiat, E Roger, K Frombach, J Gimel, P Saulnier and B Calvignac. Spontaneous nano-emulsification: Process optimization and modeling for the prediction of the nanoemulsion's size and polydispersity. *International Journal of Pharmaceutics* 2017; **534(1-2)**, 220-228.

[20] AH Saberi, Y Fang and DJ McClements. Thermal reversibility of vitamin e-enriched emulsion-based delivery systems produced using spontaneous emulsification. *Food Chemistry* 2015; **185**, 254-260.

[21] J Rao and DJ McClements. Stabilization of phase inversion temperature nanoemulsions by surfactant displacement. *Journal of Agricultural and Food Chemistry* 2010; **58(11)**, 7059-7066.

[22] D Morales, JM Gutierrez, MJ Garcia-Celma and YC Solans. A Study of the relation between bicontinuous microemulsions and oil/water nano-emulsion formation. *Langmuir* 2003; **19(18)**, 7196-7200.

[23] J Esquena and J Vilasau. Formulation, characterization, and property control of paraffin emulsions. *Emulsion Formation and Stability* 2013; **(6)**, 169-197.

[24] N Anton and TF Vandamme. Nano-emulsions and micro-emulsions: Clarifications of the critical differences. *Pharmaceutical Research* 2011; **28(5)**, 978-985.

[25] DJ McClements. Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter* 2012; **8(6)**, 1719-1729.

[26] C Solans, P Izquierdo, J Nolla, N Azemar and MJ Garcia-Celma. Nano-emulsions. *Current Opinion in Colloid & Interface Science* 2005; **10(3-4)**, 102-110.

[27] TG Mason, JN Wilking, K Meleson, CB Chang and SM Graves. Nanoemulsions: Formation, structure, and physical properties. *Journal of Physics: Condensed Matter* 2007; **18(41)**, R635-R666.

[28] SM Jafari, Y He and B Bhandari. Nano-emulsion production by sonication and microfluidization - a comparison. *International Journal of Food Properties* 2006; **9(3)**, 475-485.

[29] M Jaiswal, R Dudhe and PK Sharma. Nanoemulsion: An advanced mode of drug delivery system. *3 Biotech* 2015; **5(2)**, 123-127.

[30] P Chuesiang, U Siripatrawan, R Sanguandeekul, L McLandsborough and DJ McClements. Optimization of cinnamon oil nanoemulsions using phase inversion temperature method: Impact of oil phase composition and surfactant concentration. *Journal of Colloid and Interface Science* 2018; **514**, 208-216.

[31] MEI Badawy, ASA Saad, EHM Tayeb, SA Mohammed and AD 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* 2017; **52(12)**, 896-911.

[32] J Stetefeld, SA McKenna, TR Patel. Dynamic Light Scattering: A Practical Guide and Applications in Biomedical Sciences. *Biophysical Reviews* 2016; **8(4)**, 409-427.

[33] AH Saberi, Y Fang and DJ McClements. Formation of thermally reversible optically transparent emulsion-based delivery systems using spontaneous emulsification. *Soft Matter* 2015; **11(48)**, 9321-9329.

[34] JA Miller, PA Thompson, IA Hakim, H-HS Chow, CA Thomson. D-Limonene: A Bioactive Food Component from Citrus and Evidence for a Potential Role in Breast Cancer Prevention and Treatment. *Oncology Reviews* 2011; **5(1)**, 31-42.

[35] SCRD Silva, PM Lopes, MMBD Azevedo, DCM Costa, CS Alviano and DS Alviano. Biological activities of a-pinene and β-pinene enantiomers. *Molecules* 2012; **17(6)**, 6305-6316.

[36] F Bakkali, S Averbeck, D Averbeck and M Idaomar. Biological effects of essential oils: A review. *Food and Chemical Toxicology* 2008; **46(2)**, 446-475.

[37] J Komaiko and DJ McClements. Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. *Journal of Food Engineering* 2015; **146**, 122-128.

[38] M Guttoff, AH Saberi and DJ Mcclements. Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability. *Food Chemistry* 2015; **171**, 117-122.

[39] M Chouaibi. *Preparation of oil-in-water (O/W) clove essential oil nanoemulsion: Characterization and stability*. Elsevier Science, Amsterdam, Netherlands, 2022.

[40] VK Rai, N Mishra, KS Yadav and NP Yadav. Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications. *Journal of Controlled Release* 2018; **270**, 203-225.

[41] M Hennebelle, P Villeneuve, E Durand, J Lecomte, JV Duynhoven, A Meynier, B Yesiltas, C Jacobsen, C Berton-Carabin. Lipid Oxidation in Emulsions: New Insights from the Past Two Decades. *Progress in Lipid Research* 2024; **94**, 101275.

[42] HR Sharif, HD Goff, H Majeed, F Liu, J Nsor-Atindana, J Haider, R Liang and F Zhong. Physicochemical stability of β-carotene and α-tocopherol enriched nanoemulsions: Influence of carrier oil, emulsifier and antioxidant. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2017; **529**, 550-559.

[43] TSH Leong, TJ Wooster, SE Kentish and M Ashokkumar. Minimising oil droplet size using ultrasonic emulsification. *Ultrasonics Sonochemistry* 2009; **16(6)**, 721-727.

[44] A Gupta, HB Eral, TA Hatton and PS Doyle. Nanoemulsions: Formation, properties and applications. *Soft Matter* 2016; **12(11)**, 2826-2841.

[45] A Perazzo, V Preziosi and S Guido. Phase inversion emulsification: Current understanding and applications. *Advances in Colloid and Interface Science* 2015; **222**, 581-599.

[46] Alexandros Koulouris, Nikiforos Misailidis, Avraam Roussos, Jim Prentzas, Demetri P Petrides. Food Process Simulation and Techno-Economic Assessment in Sustainable Food Manufacturing. *Smart Food Industry: The Blockchain for Sustainable Engineering* 2024; **23**.

[47] NHC Marzuki, RA Wahab and MA Hamid. An overview of nanoemulsion: Concepts of development and cosmeceutical applications.

Biotechnology & Biotechnological Equipment 2019; **33(1)**, 779-797.

[48] SSL Tiang, LE Low, I Ali, L Zhou, B Goh, LT Gew and SY Tang. Recent advances in ultrasonic cavitation technologies for emulsion preparation: A mini review. *Current Opinion in Chemical Engineering* 2024; **45**, 101046.

[49] McClements DJ. *Food Emulsions*. CRC Press, Florida, 2004.

[50] FBD Carvalho-Guimaraes, KL Correa, TPD Souza, JRR Amado, RM Ribeiro-Costa and JOC Silva-Junior. A review of pickering emulsions: Perspectives and applications. *Pharmaceuticals* 2022; **15(11)**, 1413.