TEMPERATURE-DEPENDENT STABILITY AND KINETICS OF *Citrus x latifolia* ESSENTIAL OIL NANOEMULSIONS PREPARED BY LOW-ENERGY EMULSIFICATION

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ABSTRACT

Citrus x latifolia essential oil (CLEO) contains volatile monoterpenes with potential applications in food systems. However, its practical use is constrained by low water solubility in aqueous environments. While nanoemulsification has been widely investigated as a strategy for dispersing hydrophobic essential oils, the temperature-dependent stability and kinetic behavior of terpene-rich nanoemulsions produced by low-energy methods remain insufficiently understood. In this context, the present study investigated the formation and storage stability of CLEO oil-in-water nanoemulsions prepared by spontaneous emulsification using Tween 80 as the surfactant and propylene glycol as the co-surfactant. Specifically, the formulation consisted of 16% Tween 80, 16% propylene glycol, and 4% CLEO, resulting in nanoemulsions with mean droplet diameters of approximately 16-17 nm, a polydispersity index (PDI) of 0.2-0.3, and an optical transmittance of about 98%. These results indicate a relatively narrow droplet size distribution and high dispersion clarity. To further evaluate stability, temperature-dependent storage experiments were conducted at 10, 30, and 40 °C for 40 days to assess droplet growth behavior over time. The findings revealed progressive droplet growth with increasing temperature while maintaining a relatively narrow size distribution. Kinetic analysis further indicated apparent first-order droplet growth behavior, with the apparent rate constant increasing from 0.0012 to 0.0032 day-1 as the temperature increased from 10 to 40 °C, corresponding to an activation energy of 22.51 kJ mol-1. These results provide mechanistic insight into the temperature-dependent kinetic stability of CLEO nanoemulsions, contributing to an improved predictive understanding of nanoemulsion stability in low-energy systems.

Keywords: Citrus essential oil; nanoemulsion; spontaneous emulsification; temperature-dependent stability; kinetic behavior.

1. INTRODUCTION

Natural essential oils derived from citrus species have attracted increasing attention in food systems owing to their pronounced antimicrobial and antioxidant activities, together with their Generally Recognized as Safe (GRAS) status for use as flavoring and preservation agents. Citrus essential oils are predominantly composed of volatile monoterpenes; for instance, lemon essential oil has been reported to contain approximately 52.85% D-limonene, 14.36% p-cymene, and 13.69% β-pinene, which have shown significant radical scavenging capacity and antimicrobial effects relevant to food preservation against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella paratyphi* A [1, 2]. Nevertheless, their practical application in aqueous food matrices remains challenging due to low water solubility, high volatility, and susceptibility to oxidative degradation, all of which compromise dispersion stability and bioactive efficacy during processing and storage.

To overcome these physicochemical limitations, nanoencapsulation strategies, particularly oil-in-water (O/W) nanoemulsions, have emerged as effective delivery platforms. Nanoemulsions are typically characterized by droplet diameters below 100 nm, conferring high interfacial area and enhanced kinetic stability. Compared with conventional emulsions, reduced droplet size mitigates gravitational separation and coalescence while limiting Ostwald ripening through improved interfacial coverage [3]. Importantly, nanoemulsified citrus essential oils have demonstrated enhanced antimicrobial performance relative to their non-encapsulated counterparts, including stronger inhibitory effects against S. aureus and Salmonella enterica, attributable to improved dispersion and interfacial interaction with microbial membranes [1]. Consequently, nanoemulsion-based delivery systems have emerged as promising strategies for incorporating volatile essential oils into aqueous food products.

Despite these advantages, predicting the storage stability of nanoemulsions remains a major challenge in colloid and food science. Nanoemulsions are thermodynamically unstable systems that may undergo several destabilization pathways during storage, including flocculation, coalescence, and diffusion-driven droplet growth [4]. In nanoemulsions containing terpene-rich essential oils, the relatively small molecular size and partial water solubility of monoterpenes such as limonene and pinene can facilitate molecular diffusion through the aqueous phase, potentially accelerating droplet growth processes associated with Ostwald ripening [5]. Temperature further influences these molecular transport phenomena by modifying oil solubility, diffusion coefficients, and interfacial dynamics, which ultimately affect droplet growth kinetics and nanoemulsion shelf-life stability. However, the physicochemical mechanisms governing temperature-dependent instability behavior in terpene-rich nanoemulsions remain insufficiently understood. In particular, the ability to predict droplet growth kinetics and identify the dominant instability pathway in such systems remains limited.

Although numerous studies have investigated the formulation and antimicrobial performance of essential-oil nanoemulsions, most research has focused primarily on droplet size optimization or bioactivity evaluation. Mechanistic analyses linking compositional phase behavior, processing conditions, and temperature-dependent droplet growth kinetics remain relatively scarce, particularly for nanoemulsions produced using low-energy emulsification approaches. Low-energy methods such as spontaneous emulsification rely mainly on interfacial tension reduction and surfactant self-assembly rather than mechanical disruption, leading to distinct droplet formation pathways and potentially different stability behavior compared with high-energy homogenization techniques [6]. Consequently, integrated investigations that simultaneously evaluate phase behavior, processing variables, and temperature-dependent stability kinetics are still limited for terpene-rich citrus essential oil systems. This lack of systematic analysis represents an important knowledge gap in understanding the molecular transport processes governing droplet growth and in establishing predictive frameworks for nanoemulsion stability in food applications.

Based on these considerations, the objective of this study was to develop a stable O/W nanoemulsion containing CLEO using a spontaneous emulsification strategy with tween 80 and propylene glycol (PG). The formulation domain capable of producing nanoemulsions was first identified through pseudo-ternary phase behavior analysis. Subsequently, the influence of key processing parameters on droplet size was systematically evaluated. Finally, temperature-dependent droplet growth during storage was analyzed using kinetic modeling and Arrhenius analysis to estimate the apparent activation energy and infer the dominant instability mechanism. By integrating formulation design with temperature-dependent kinetic analysis, this study aims to provide mechanistic insight into instability behavior and improve the predictability of stability in terpene-rich nanoemulsion systems.

2. MATERIALS AND METHODS

2.1. Materials

CLEO was provided by Chanh Viet Long An Trading and Investment JSC. (Tay Ninh province, Viet Nam) and stored under refrigerated conditions until use. Tween 80 (CAS 9005-65-6, extra pure) was sourced from Fisher Scientific (Waltham, Massachusetts, USA). PG (CAS 57-55-6) was obtained from Guangdong Guanghua Sci-Tech C., LTD. (Guangdong, China). Distilled water, produced in-house, served as the aqueous phase in all experiments.

2.2. Nanoemulsion preparation

Nanoemulsions were prepared using a low-energy spontaneous emulsification method. A surfactant mixture (Smix) consisting of Tween 80 and PG at a fixed weight ratio of 1:1 was first prepared. The required amount of Smix was dispersed in distilled water under magnetic stirring (VELP Scientifica HSC, Italy) until a clear and uniform solution was obtained. Subsequently, the CLEO was added dropwise into the aqueous Smix phase under continuous stirring to promote spontaneous nanoemulsion formation.

For formulation screening, CLEO concentration was varied from 4 to 10% (w/w), while the mass ratio of CLEO to Smix was progressively increased from 1:1 upward. The combined concentration of CLEO and Smix did not exceed 50% (w/w), and water content was adjusted accordingly to complete 100% (w/w), yielding 24 formulations (Table 1). All samples were prepared under identical processing conditions and equilibrated prior to visual assessment. Systems were classified as phase-separated, turbid homogeneous, or transparent homogeneous.

Table 1. Formulation screening matrix for nanoemulsion development

CLEO (%, w/w)	Tween 80 (%, w/w)	PG (%, w/w)	Water (%, w/w)	CLEO (%, w/w)	Tween 80 (%, w/w)	PG (%, w/w)	Water (%, w/w)
10	5	5	80	6	12	12	70
10	10	10	70	6	15	15	64
10	15	15	60	6	18	18	58
10	20	20	50	6	21	21	52
8	4	4	84	4	2	2	92
8	8	8	76	4	4	4	88
8	12	12	68	4	6	6	84
8	16	16	60	4	8	8	80
8	20	20	52	4	10	10	76
6	3	3	88	4	12	12	72
6	6	6	82	4	14	14	68
6	9	9	76	4	16	16	64

The compositional data were plotted on a pseudo-ternary phase diagram (CLEO-Smix-water) to identify the nanoemulsion region. The suitable formulation selected from the transparent isotropic region was subsequently subjected to process optimization by varying stirring speed (200-400-600 rpm) and stirring time (10-20-30 min), followed by storage stability evaluation at different temperatures.

2.3. Storage stability

The stability of CLEO nanoemulsion was assessed under various storage temperatures. Initially, 50 g of nanoemulsion was packed into individual glass bottles, sealed, and then stored at 10, 30, and 40 °C for a duration of 40 days. Changes in droplet size, PDI and transmittance were monitored on day 0, 10, 20, 30 and 40. The degradation of these parameters was calculated by using the standard equation for a first order kinetic model:

$$\ln C = \ln C_o - k(t)$$

The half-life ($t_{1/2}$) was computed at a specific temperature using the equation:

$$t_{1/2} = \ln 2 / k$$

The activation energy (Ea) was determined utilizing the Arrhenius equation:

$$k = A \times e^{\frac{-E_a}{RT}}$$

where C denotes the value at time t; Co signifies the value at the initial time; k is the degradation rate constant (day^{-1}), derived from the slope of a plot of the natural log of C/Co against time; t represents the storage duration (days); R is the gas constant (8.3145 J/K mol); T indicates the temperature in Kelvin; A (L/mol/s) is the frequency factor, considering the frequency of reactions and probability of correct molecular orientation.

2.4. Particle size and Polydispersity index (PDI)

The mean droplet diameter and PDI of the nanoemulsions were determined by dynamic light scattering (DLS) using a nanoparticle analyzer (SZ-100, HORIBA, Japan) at 25 ± 0.1 °C. The scattered light intensity fluctuations, generated by the Brownian motion of droplets, were recorded at a fixed detection angle and analyzed to calculate the translational diffusion coefficient and corresponding hydrodynamic diameter. The reported particle size corresponds to the intensity-weighted Z-average diameter obtained from the autocorrelation function of the scattered light signal. Measurements were performed in general mode with a data acquisition time of 90 s, and each sample was analyzed in triplicate.

2.5. Transmittance measurement

The optical transparency of the nanoemulsions was evaluated by measuring percent transmittance at 650 nm using a UV-Vis spectrophotometer (Jasco - V730, Japan), with distilled water as the blank, following the method described by Christy et al. [7]. All measurements were performed in triplicate.

2.6. Statistical analysis

All experiments and corresponding analyses were conducted in triplicate. Significant differences between means at $p<0.05$ were determined using the SPSS software version 23.0 statistical package (IBM Australia Limited, St Leonard, NSW, Australia). Pearson correlation coefficients were also calculated using the SPSS software.

3. RESULTS AND DISCUSSION

3.1. Formulation design and phase behavior of CLEO nanoemulsion

The formation of stable O/W nanoemulsions is strongly influenced by the balance of components and the interfacial phenomena determined by their composition. In this system, it may be related to the molecular properties of terpenes in the essential oil, such as limonene or β-caryophyllene. Terpene compounds typically have low molecular weights and low oil-phase viscosity, making the oil phase more susceptible to breaking down into smaller droplets during emulsification. Many studies have shown that essential oils containing small molecules and low viscosity tend to form nanoemulsions with smaller droplet sizes compared to oils with higher viscosity, due to their lower droplet breakage resistance and more efficient dispersion [8].

In addition, Tween 80 plays a significant role in the formation of very small nanoparticles in the system. Tween 80 is a non-ionic surfactant with a high HLB value (~15), suitable for O/W emulsion systems, with a hydrophilic polyoxyethylene chain and a hydrophobic oleate tail capable of strong adsorption at the oil-water interface. When combined with PG, small co-surfactant molecules can insert into the interfacial layer formed by Tween 80, increasing the mobility of the surfactant film and reducing the stiffness of the adsorbent layer. This combination reduces interfacial tension, allowing the system to achieve higher surface curvature and facilitating the formation of very small and more stable nano-sized droplets in the nanoemulsion system [7, 9]. In this study, the relatively high surfactant concentration was employed to expand the nanoemulsion region in the pseudo-ternary phase diagram and facilitate spontaneous emulsification. Therefore, the formulations in this study should primarily be regarded as a screening system to identify the nanoemulsion region, providing a basis for subsequent researches toward formulations with lower surfactant levels for practical applications.

The pseudo-ternary phase diagram (Figure 1) clearly showed that increasing the surfactant mixture fraction expanded the transparent monophasic region, similar to the research on pepper essential oil emulsion system by Hien et al. [10]. According to classic emulsification theory, surface-active agents reduce the interfacial tension between two immiscible phases by preferentially adsorbing at the interface, thereby lowering the work required to create new surface area and facilitating the breakdown of the dispersed phase into fine droplets [11]. By decreasing interfacial free energy, surfactants promote the generation of small droplets with a reduced tendency to re-agglomerate, which subsequently allows the formation of a stable dispersion with high optical clarity. The observed expansion of the transparent region in the phase diagram is therefore indicative of efficient interfacial stabilization through surfactant action, consistent with established surface tension reduction mechanisms in nanoemulsion formation. Such behavior aligns with the fundamental understanding of low-energy spontaneous emulsification, where nano-scale droplets are generated through intrinsic interfacial processes without the need for high external energy input, as gentle mixing allows surfactant molecules to distribute and diffuse at the interface and drive the self-assembly of dispersed domains [12].

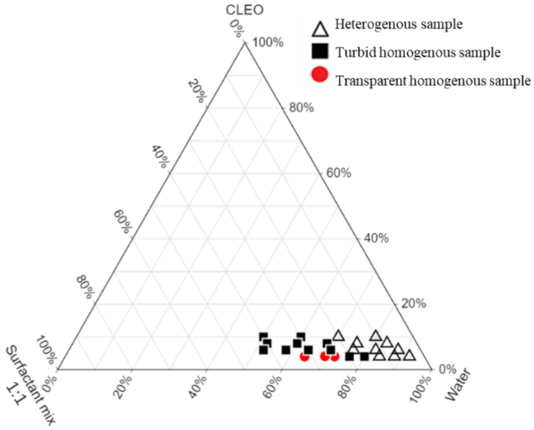

Figure 1. Pseudo-ternary phase diagram of CLEO-surfactant mixture-water

Table 2. Droplet sizes, PDI, and transmittance of CLEO nanoemulsion formed by different formulas

No.	CLEO (%, w/w)	Tween 80 (%, w/w)	PG (%, w/w)	Water (%, w/w)	Droplet size (nm)	PDI	Transmittance (%)
1	4	12	12	72	117.67 ± 3.00^a	0.39 ± 0.04^a	92.83 ± 0.31^c
2	4	14	14	68	83.70 ± 2.03^b	0.27 ± 0.07^b	96.10 ± 1.25^b
3	4	16	16	64	16.80 ± 0.30^c	0.26 ± 0.05^b	98.77 ± 0.61^a
a, b, and c superscript of each value in the same column denotes the statistically significant difference at $p < 0.05$							

The formulations that produced a homogeneous, transparent emulsion system, as observed visually, were further analyzed for particle size, PDI, and transmittance to select the most suitable formulation. The results shown in Table 2 once again emphasize that the higher the surfactant ratio, the greater the amount of surfactant molecules diffusing from the organic phase to the aqueous phase, and the stronger the adsorption of surfactant molecules onto the interface between the two phases, thus reducing surface tension and facilitating the formation of smaller emulsion droplets [13, 14]. In addition, the tendency for transmittance to increase is explained by the fact that when the size of the dispersed particles is smaller than the wavelength of light (usually <100 nm), the transmitted light is less scattered, causing the system to change from a milky opaque to transparent. Hence, No.3 was selected as the most suitable formulation for further investigation.

3.2. Effect of processing parameters on droplet refinement

Spontaneous emulsification represents a low-energy approach in which stirring plays a critical role in facilitating uniform mass transfer and continuous surface renewal. As presented in Table 3, increasing the stirring speed from 200 to 600 rpm resulted in a statistically significant reduction in droplet size, from approximately 42 nm to 16 nm ($p<0.05$). This observation aligns with the findings of Barzegar et al. [15], who reported that elevating the stirring speed from 500 to 900 rpm in peppermint nanoemulsions decreased droplet size from 112 nm to 41 nm. Notably, both the PDI and transmittance remained unaffected, indicating no significant variation across speeds conditions.

Table 3. Droplet sizes, PDI, and transmittance of CLEO nanoemulsion at different stirring speeds

Stirring speed (rpm)	Droplet size (nm)	PDI	Transmittance (%)
200	42.03 ± 0.76^a	0.28 ± 0.06	98.21 ± 1.09
400	27.07 ± 1.31^b	0.32 ± 0.05	98.42 ± 0.89
600	16.53 ± 0.81^c	0.28 ± 0.06	98.97 ± 1.08
Values are mean ± SD (triplicates) after statistical analyses. a, b, and c superscript of each value in the same column denotes the statistically significant difference at $p < 0.05$.			

While, Table 4 demonstrates a clear time-dependent refinement of droplet size during spontaneous emulsification. Extending the stirring duration from 10 to 30 minutes reduced the mean droplet diameter from approximately 22 nm to approximately 17 nm, which showed a pathway in which progressive surfactant adsorption and continued mass transfer at the oil-water interface lower interfacial tension and shift the preferred curvature toward smaller radii, thereby enabling the nucleation and stabilization of finer droplets [16].

Table 4. Droplet sizes, PDI, and transmittance of CLEO nanoemulsion at different stirring times

Stirring time (min)	Droplet size (nm)	PDI	Transmittance (%)
10	22.77 ± 1.48^{a}	0.22 ± 0.04	98.30 ± 0.95
20	20.60 ± 1.49^{b}	0.21 ± 0.02	98.93 ± 0.67
30	17.00 ± 0.72^{c}	0.20 ± 0.04	99.03 ± 0.23

Values are mean ± SD (triplicates) after statistical analyses. a, b, and c superscript of each value in the same column denotes the statistically significant difference at $p < 0.05$.

Although stirring speed and stirring time were evaluated in two independent experiments, both parameters influence nanoemulsion formation through related interfacial and mass-transfer mechanisms inherent to spontaneous emulsification. The influence of stirring speed on droplet size and dispersion behavior has been widely documented [17, 18]. Increasing agitation intensity generates stronger shear forces and turbulence, which expands the interfacial contact area between the oil and aqueous phases and enhances convective transport. This promotes a more homogeneous redistribution of surfactant molecules at the interface, leading to a reduction in interfacial tension and facilitating droplet fragmentation into smaller domains [19-21]. Consequently, higher stirring speeds primarily accelerate the physical dispersion of the oil phase and improve the efficiency of interfacial mixing during the early stages of emulsification.

Besides, the effect of stirring time reflects the progressive approach of the system toward interfacial and compositional equilibrium during the spontaneous emulsification process. The time-dependent reduction in droplet size can be explained by two coupled phenomena. First, surfactant adsorption kinetics govern droplet stabilization: at short processing times, incomplete interfacial coverage maintains relatively high interfacial tension, resulting in larger droplets. As adsorption proceeds and surface saturation approaches equilibrium, interfacial tension decreases, enabling further droplet size reduction. Second, diffusion-driven mass transfer between phases, including solvent exchange and compositional equilibration, gradually modifies interfacial composition and chemical potential gradients, thereby promoting the nucleation and structural refinement of dispersed droplets. Although stirring enhances convective transport and accelerates surfactant redistribution, the attainment of equilibrium interfacial coverage and full phase diffusion remains intrinsically time-controlled in low-energy emulsification systems. Consistent with this interpretation, the PDI and transmittance were not significantly affected by stirring time ($p > 0.05$), with transmittance remaining above 98% across conditions, indicating that droplet refinement occurred without aggregation or loss of optical clarity.

From a practical standpoint, emulsification conditions must balance efficient droplet size reduction with preservation of essential oil integrity. Insufficient processing leads to incomplete dispersion and larger droplets, whereas excessive processing may increase the risk of oxidative degradation of volatile oil components. Considering the observed reduction in droplet size together with the stable polydispersity index and transmittance, a stirring speed of 600 rpm and an emulsification time of 30 min were selected as appropriate conditions for subsequent experiments.

3.3. Storage stability

3.3.1. Physical stability behavior

The physical stability of the CLEO nanoemulsion during storage was evaluated by monitoring droplet size evolution under 10, 30, and 40 °C over 40 days. As shown in Table 5-7, a gradual increase in mean droplet diameter was observed over time, and the rate of droplet growth increased with storage temperature. Samples stored at higher temperatures exhibited a more pronounced increase in droplet size compared with those stored at lower temperatures, indicating that temperature significantly influenced the destabilization kinetics of the system. The more pronounced growth at 40 °C is consistent with diffusion-driven destabilization, particularly Ostwald ripening, in which curvature-induced Laplace pressure differences promote molecular transfer from smaller to larger droplets [22-24]. According to Lifshitz-Slyozov-Wagner theory, ripening rates increase with temperature due to enhanced diffusion coefficients and dispersed-phase solubility. Notably, mean diameters remained below 20 nm throughout storage, preserving nanoscale dispersion and preventing creaming or visible turbidity.

Table 5. Physicochemical stability of CLEO nanoemulsion during storage at 10 °C

Storage time (day)	Droplet size (nm)	PDI	Transmittance (%)
0	16.10 ± 0.03^{a}	0.22 ± 0.01	98.30 ± 0.20
10	16.10 ± 0.02^{a}	0.25 ± 0.05	98.30 ± 0.36
20	16.40 ± 0.01^{b}	0.27 ± 0.08	98.27 ± 0.21
30	16.69 ± 0.04^{c}	0.22 ± 0.03	98.37 ± 0.31
40	16.80 ± 0.01^{c}	0.27 ± 0.07	98.57 ± 0.31
a, b, and c superscript of each value in the same column denotes the statistically significant difference at p<0.05			

Table 6. Physicochemical stability of CLEO nanoemulsion during storage at 30 °C

Storage time (day)	Droplet size (nm)	PDI	Transmittance (%)
0	16.10 ± 0.03^{a}	0.22 ± 0.01	98.30 ± 0.20
10	16.61 ± 0.01^{b}	0.22 ± 0.05	98.03 ± 0.32
20	16.89 ± 0.03^{c}	0.26 ± 0.05	98.57 ± 0.35
30	17.12 ± 0.02^{d}	0.22 ± 0.02	98.53 ± 0.49
40	17.41 ± 0.01^{e}	0.27 ± 0.06	98.40 ± 0.43
a, b, c, d and e superscript of each value in the same column denotes the statistically significant difference at p<0.05			

Table 7. Physicochemical stability of CLEO nanoemulsion during storage at 40 °C

Storage time (day)	Droplet size (nm)	PDI	Transmittance (%)
0	16.10 ± 0.03^{a}	0.22 ± 0.01	98.30 ± 0.20
10	16.89 ± 0.01^{b}	0.22 ± 0.05	98.03 ± 0.32
20	17.71 ± 0.02^{c}	0.26 ± 0.05	98.57 ± 0.35
30	17.80 ± 0.02^{d}	0.22 ± 0.02	98.53 ± 0.49
40	18.39 ± 0.01^{e}	0.27 ± 0.06	98.40 ± 0.43
a, b, c, d and e superscript of each value in the same column denotes the statistically significant difference at p<0.05			

Despite the progressive increase in droplet size, the narrow PDI range of 0.22-0.27 and high transmittance of approximately 98% remained relatively stable throughout the storage period. Uniform size enlargement without distribution broadening contrasts with the abrupt growth and heterogeneity typical of coalescence or flocculation, suggesting that the observed changes primarily reflect gradual droplet coarsening rather than macroscopic destabilization of the system [24]. Moreover, because the droplet diameters were substantially smaller than the wavelength of visible light, light scattering occurred within the Rayleigh regime. Under these conditions, although scattering intensity is proportional to the sixth power of particle diameter, the absolute scattering remains very low, thereby helping to maintain high optical clarity. Consequently, slight size increases did not induce optical attenuation.

3.3.2. Kinetic modelling

To quantitatively describe the droplet growth behavior, the experimental data were fitted to a first-order kinetic model. The strong linear relationship between ln(size/initial size) and storage time (Figure 2), was obtained across the tested temperatures ($R^2 > 0.93$), indicating that droplet growth followed apparent first-order kinetics. The kinetic rate constant increased progressively with temperature, ranging from 0.0012 day^{-1} at 10 °C to 0.0032 day^{-1} at 40 °C (Figure 3), demonstrating that elevated temperatures accelerated droplet growth during storage.

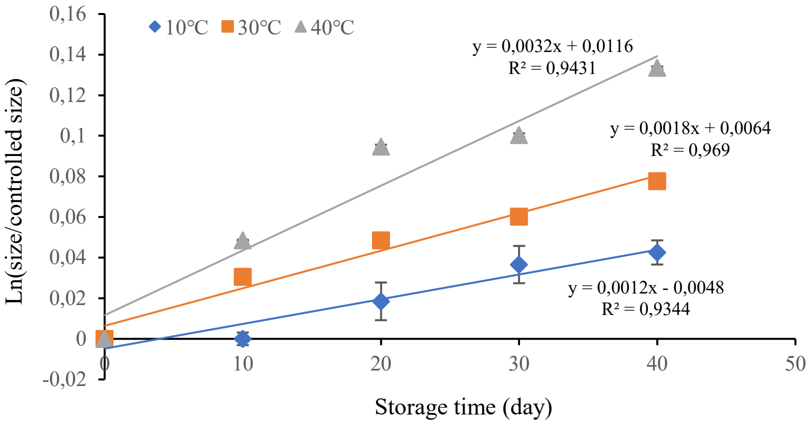

Figure 2. The first-order degradation kinetics of nanoemulsion formulation at different temperatures

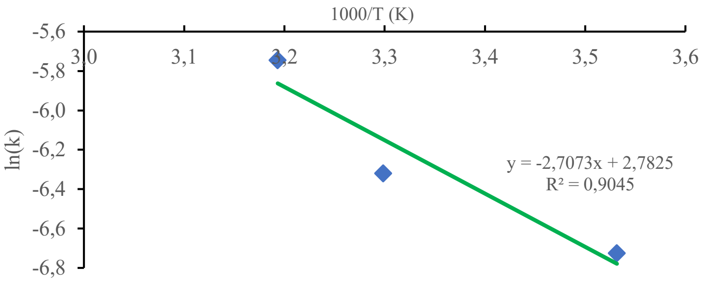

Figure 3. Arrhenius plot between ln(k) and 1/T*10^3 (K) of CLEO nanoemulsion

Table 8. Half-life of nanoemulsion at different temperatures

Temperatures			Degradation rate k (day^{-1})	Half-life (day)	Ea (kJ·mol^{-1})
°C	K	1000/T (K)			
10	283.15	3.532	0.0012	577.62	22.51
30	303.15	3.299	0.0018	385.08	
40	313.15	3.193	0.0032	216.61	

The temperature dependence of the kinetic rate constants was further evaluated using the Arrhenius equation. The Arrhenius plot exhibited good linearity, allowing estimation of the apparent activation energy for droplet growth. The calculated activation energy was 22.51 kJ·mol^{-1} (Table 8) is comparable to that reported for a garlic essential oil nanoemulsion system, which exhibited an Ea of 28.38 kJ·mol^{-1} over 5 to 45 °C [25], indicating a moderate sensitivity of the destabilization process to temperature. This magnitude lies within the range typically associated with diffusion controlled ripening in fluid nanoemulsion systems. The determination of temperature-dependent kinetic parameters provides a quantitative framework for evaluating nanoemulsion stability and contributes to a predictive understanding of droplet growth behavior under different storage conditions.

3.3.3. Mechanistic interpretation

The kinetic characteristics observed in this study provide insight into the underlying destabilization mechanism of the CLEO nanoemulsion during storage. Although increasing temperature accelerated droplet growth, the kinetic pattern and size distribution remained consistent across conditions, indicating that thermal stress intensified molecular transport without altering the underlying destabilization pathway. The absence of abrupt size jumps or multimodal distributions suggests a diffusion mediated mechanism rather than collision induced coalescence. Such kinetic regularity is widely associated with Ostwald ripening controlled systems [26, 27].

In addition, the relatively low apparent activation energy obtained from Arrhenius analysis suggests that the rate-limiting step of droplet growth is associated with molecular diffusion rather than interfacial rupture or collision-induced coalescence. Processes such as coalescence typically require disruption of the surfactant-stabilized interfacial film and therefore exhibit higher activation energies due to the energetic barrier associated with interfacial deformation and film drainage. In contrast, diffusion-controlled growth mechanisms, including Ostwald ripening or molecular exchange between droplets, are primarily governed by the temperature dependence of molecular diffusivity and oil solubility in the continuous phase [27, 28].

Taken together, the combined evidence from physicochemical parameters, kinetic modeling, Arrhenius analysis, and activation energy comparison consistent with Ostwald ripening is the dominant long term destabilization mechanism in this CLEO nanoemulsion, while coalescence plays a negligible role under the investigated storage conditions.

4. CONCLUSION

The present study demonstrates that spontaneous emulsification can generate highly transparent CLEO oil-in-water nanoemulsions with droplet diameters in the nanometric range (~16-17 nm) and narrow size distributions (PDI ~0.2), confirming the suitability of low-energy approaches for dispersing terpene-rich essential oils in aqueous environments. Temperature-dependent storage analysis further revealed a consistent and predictable droplet growth pattern, in which the apparent kinetic rate constant increased from 0.0012 to 0.0032 day^{-1} between 10 and 40 °C, corresponding to an activation energy of 22.51 kJ mol^{-1}. The relatively low activation barrier, together with the gradual and continuous increase in droplet size, provides mechanistic evidence that droplet coarsening in terpene-rich nanoemulsions produced via low-energy emulsification follows a thermally activated diffusion pathway.

More broadly, integrating temperature-dependent kinetic analysis with formulation optimization provides a useful framework for developing a predictive understanding of nanoemulsion stability. Such an approach contributes to the mechanistic interpretation of instability pathways in terpene-rich nanoemulsions and may support the rational design of essential-oil delivery systems where temperature variations influence long-term structural stability.

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TÓM TẮT

ẢNH HƯỞNG CỦA NHIỆT ĐỘ ĐẾN ĐỘ ỔN ĐỊNH VÀ ĐỘNG HỌC CỦA NHŨ TƯƠNG NANO TINH DẦU CHANH (*Citrus x latifolia*) SỬ DỤNG PHƯƠNG PHÁP NHŨ HÓA NĂNG LƯỢNG THẤP

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Tinh dầu *Citrus x latifolia* (CLEO) chứa các monoterpen dễ bay hơi có tiềm năng ứng dụng trong hệ thống thực phẩm. Tuy nhiên, ứng dụng thực tế của nó bị hạn chế bởi độ hòa tan thấp trong môi trường nước. Mặc dù hệ nhũ tương nano đã được nghiên cứu rộng rãi như một chiến lược để phân tán các loại tinh dầu kỵ nước, nhưng tính ổn định phụ thuộc vào nhiệt độ và hành vi động học của các nhũ tương nano giàu terpen được tạo ra bằng các phương pháp nhũ hóa năng lượng thấp vẫn chưa được nghiên cứu sâu. Trong bối cảnh này, nghiên cứu hiện tại đã khảo sát sự hình thành và độ ổn định khi bảo quản của nhũ tương nano dầu trong nước (O/W) được điều chế bằng phương pháp nhũ hóa tự phát sử dụng Tween 80 làm chất hoạt động bề mặt và propylene glycol (PG) làm chất đồng hoạt động bề mặt. Cụ thể, công thức bao gồm 16% Tween 80, 16% PG và 4% CLEO, tạo ra các nhũ tương nano có đường kính hạt trung bình khoảng 16-17 nm, chỉ số phân tán (PDI) là 0,2-0,3 và độ truyền quang khoảng 98%. Những kết quả này cho thấy sự phân bố kích thước hạt tương đối hẹp và độ trong suốt phân tán cao. Để đánh giá thêm về độ ổn định, các thí nghiệm bảo quản đã được tiến hành ở 10, 30 và 40 °C trong 40 ngày để đánh giá hành vi tăng trưởng giọt theo thời gian dưới sự tác động của nhiệt độ. Kết quả cho thấy sự tăng trưởng kích thước hạt dần dần khi nhiệt độ tăng trong khi vẫn duy trì PDI tương đối hẹp. Phân tích động học cho thấy hành vi tăng trưởng kích thước theo hàm bậc nhất, với hằng số tốc độ biểu kiến tăng từ 0,0012 đến 0,0032 ngày^{-1} khi nhiệt độ tăng từ 10 đến 40 °C, tương ứng với năng lượng hoạt hóa là 22,51 kJ mol^{-1}. Những kết quả này cung cấp cái nhìn sâu sắc về cơ chế ổn định động học phụ thuộc vào nhiệt độ của nhũ tương nano CLEO, góp phần cải thiện khả năng dự đoán độ ổn định của nhũ tương nano trong các hệ thống năng lượng thấp.

Từ khóa: Tinh dầu chanh, nhũ tương nano, nhũ hóa tự phát, độ ổn định phụ thuộc vào nhiệt độ, mô hình động học.