Food Bioscience 68 (2025) 106526

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Characterization, antibacterial activity, and stability of supercritical fluid extracted lemongrass nanoemulsion on *Bacillus cereus*

Ili Syuhada Mohd Daud [a,*], Nor Khaizura Mahmud Ab Rashid [b], Jon Palmer [a], Steve Flint [a]

[a] School of Food Technology and Natural Sciences, Massey University, Private Bag 11222, Palmerston North, 4442, New Zealand
[b] Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia

ARTICLE INFO

Keywords:
Supercritical fluid extraction
Lemongrass
Nanoemulsion
Bacillus cereus
Antimicrobial activity
Stability

ABSTRACT

Natural food preservation is a sustainable approach to extend shelf life, combat foodborne pathogens and enhance food safety. *Bacillus cereus*, a resilient contaminant, poses challenges due to its spore-forming ability and association with foodborne illnesses. This study investigates the characterization, antimicrobial activity, and stability of lemongrass (*Cymbopogon citratus*) nanoemulsions, extracted using supercritical fluid extraction (SFE), and their efficacy against *B. cereus* isolates (ATCC 14579, P4, and M2). Lemongrass oil was extracted at 85, 100, 200, and 300 bar, with the highest yield (0.815 %) obtained at 300 bar. Nanoemulsions were formulated with lemongrass extract and commercial citral, characterized for droplet size, polydispersity index (PDI), conductivity, and zeta potential, and assessed for antimicrobial activity. Lemongrass nanoemulsions initially had droplet sizes of 86.32 ± 0.66 nm, but increased over six months due to coalescence, with PDI values rising from 0.50 ± 0.00 to 0.81 ± 0.27, indicating reduced stability. Although zeta potential declined from −44.01 ± 1.69 mV to −33.63 ± 1.45 mV, it remained within the stable range (>±30 mV), maintaining sufficient electrostatic repulsion to prevent rapid aggregation. At 2.0 % concentration, nanoemulsions effectively suppressed *B. cereus* isolates (<1.00 CFU/mL), though efficacy declined after four months with increasing droplet size. Lemongrass nanoemulsions exhibited comparable antibacterial activity and stability trends to citral, suggesting that whole lemongrass extract retains its bioactivity as effectively as its major compound. Improved stabilization strategies, such as polymer encapsulation, could enhance shelf life, expanding applications in food preservation.

1. Introduction

Utilizing plant extracts as a natural food preservative has become an increasing trend as relatively cheap, readily available, environmentally friendly, natural compounds attractive to consumers and food manufacturers to enhance food safety and reduce food waste. As consumers demand healthier, less chemically processed food, plant-based preservatives present a promising alternative to synthetic chemicals. Among these, lemongrass (*Cymbopogon citratus*) stands out due to its notable antimicrobial properties, primarily attributed to its high citral content, a compound recognized for its bactericidal efficacy against various foodborne pathogens and fungi including *Bacillus cereus* (Gao et al., 2020). *B. cereus*, a spore-forming bacterium, is particularly challenging in food safety due to its ability to survive extreme environmental conditions, including heat, and its association with foodborne illnesses (Guo et al., 2021). It is known also as a significant biofilm former in dairy plants, posing a concern due to their resilience and prevalence in various milk products (Fei et al., 2019; Radmehr et al., 2020; Yang et al., 2023).

Lemongrass, beyond its antimicrobial capacity, offers other advantages when applied in food preservation. Faheem et al., (2022) highlighted that besides antimicrobial activity, lemongrass essential oil also possesses antioxidant, antifungal, and anti-insecticidal properties, making it an excellent candidate for natural food preservative. It can be extracted using non-thermal techniques, such as supercritical fluid extraction, which preserve bioactive compounds more effectively than traditional thermal methods (Moreira et al., 2019). Nejia et al., (2013) found that compared to hydrodistillation, supercritical fluid extraction produces 34 % more essential oil and keeps more natural scents and compounds of *Cupressus sempervirens*. Supercritical fluid extraction of extraction *Pimenta Racemosa* performs better than steam distillation as it yields higher phenolic content in less time and offers greater flexibility for extracting diverse bioactive compounds (McGaw et al., 2016). With its high yield, enhanced selectivity, and stability, supercritical fluid

* Corresponding author.
E-mail address: I.S.MohdDaud@massey.ac.nz (I.S. Mohd Daud).

https://doi.org/10.1016/j.fbio.2025.106526
Received 6 February 2025; Received in revised form 26 March 2025; Accepted 2 April 2025
Available online 2 April 2025

extraction is now a preferred green extraction method in pharmaceuticals, food, and cosmetics (Zhang et al., 2018). This preservation approach aligns well with the food industry's shift toward minimizing thermal processing to maintain the quality, nutritional profile, and functionality of natural preservatives (Herzyk et al., 2024). The incorporation of such compounds in nanoemulsion form is increasingly recognized for enhancing stability, bioavailability, and controlled release, which may further extend shelf life and stability under various storage conditions (Barradas & de Holanda e Silva, 2020).

Nanoemulsions offer an advanced delivery system for essential oils like lemongrass, providing better stability against environmental factors such as light, oxygen, and temperature fluctuations (Liu et al., 2019). The nanoemulsion approach not only helps improve the longevity of active compounds but also strengthen their antimicrobial potential by facilitating more effective interactions with microbial cell walls (Mushtaq et al., 2023). The studies by da Silva Gundel et al., (2018) and Gago et al., (2019) demonstrated that lemongrass nanoemulsions significantly enhance the stability and antimicrobial activity of lemongrass oil, effectively targeting pathogens such as *Pseudomonas*, *Staphylococcus aureus*, and *Escherichia coli*. However, these studies did not focus on the stability or efficacy of lemongrass nanoemulsions specifically against *B. cereus*, leaving a critical gap in the understanding of their potential application for combating this resilient foodborne pathogen.

This study aims to investigate the physicochemical characterization, storage stability, and antimicrobial efficacy of supercritical fluid-extracted lemongrass nanoemulsions and commercial sample of citral on *B. cereus*, with the goal of advancing food preservation methods that are both safe and effective. Ultimately, this research seeks to contribute to the development of sustainable natural food preservatives, providing a foundation for future studies on nanoemulsions of plant-based extracts as viable alternatives to synthetic additives in food preservation.

2. Materials and methods

2.1. Sample

Raw lemongrass was purchased from farmer in Lenggeng, Negeri Sembilan Malaysia and the plant taxonomic identification was verified in Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) under the voucher specimen number of MFI 0217/21. A commercial sample of citral, (Sigma-aldrich CAS number: 5392-40-5, MO, USA) a pure compound, is utilized as a benchmark to evaluate and compare its activity against lemongrass extract.

2.2. Extract preparation

Lemongrass leaves and stems were chopped to an average of 1 cm in size, dried in a forced air convection oven at 50 °C overnight and were ground through a dry blender until the size was not less than 100 μm in size.

2.3. Supercritical fluid extraction

About 170 g of dried and ground samples were loaded in the supercritical fluid extractor (SFE420-50-200, Feyecon Development, and Implementation B.V, Netherlands). The average CO_2 flow rate was 1.8–2.0 kg/h, and the following extraction conditions were employed: 85 bar and 40 °C; 100 bar and 40 °C; 200 bar and 40 °C; 300 bar and 40 °C. Each extraction experiment was carried out for a period of 7 h. The stock solution was kept at −20 °C for a maximum of 24 months; however, prolonged storage may lead to gradual degradation of bioactive compounds.

2.4. Yield of lemongrass

The yield of lemongrass extract was calculated using the formula of yield percentage : Yield (%) = (Weight of extracted sample/Initial weight of sample) × 100.

2.5. Identification and quantification of active compounds in lemongrass extract using gas chromatography mass spectrometry (GCMS)

Lemongrass essential oil extract was dissolved in ethanol High Performance Liquid Chromatography (HPLC) grade (R & M Marketing, Essex, UK) to yield 5 mg/mL an aliquot of the extract was injected into a QP2010 Ultra gas chromatograph-mass spectrometer (Shimadzu Corporation, Kyoto, Japan) with a BP5MS column (30 m × 0.25 mm × 0.25 μm) for compound separation. Helium was used as the carrier gas at a flow rate of 3 mL/min. The oven injector temperature was 250 °C; source temp 200 °C. The oven temperature was 50 °C, ramped up to 300 °C at 15 °C/min to and held for 10 min. The peaks were analyzed by comparing their retention times and mass fragments patterns with standard spectra available in the Shimadzu GCMS NIST/Wiley library.

2.6. Primary emulsion formation

A sodium alginate aqueous solution was made by dissolving 2 % sodium alginate (w/w) (Ajax Finechem, Auckland, New Zealand) in miliQ water at 70 °C with continuous overnight stirring until it was completely dissolved. Then, it was left to cool at 21 °C. The primary emulsion was formed by mixing the sodium alginate aqueous solution with 2 % of lemongrass extract or citral and Tween 80 with a laboratory blender (T-25 digital Ultraturrax IKA, Staufen, Germany Ultraturrax) at 13 500 rpm for 2–3 min with 30 s intervals. The volume of Tween 80 used was 1:3 ratio of extract and Tween 80 following methodology proposed by (Gago et al., 2019).

2.7. Nanoemulsion formation

The primary emulsions were passed through the microfluidization process (M-110P, Microfluidics, USA) at 150 MPa for 3 cycles to obtain the nanoemulsions. The external coil of the microfluidizer was immersed in ice so that the nanoemulsions were cooled down at the outlet of the microfluidization unit and the temperature was kept at 10 °C. Nanoemulsions were kept in capped plastic tubes and stored at 4 °C room in the absence of light.

2.8. Nanoemulsions characterization

2.8.1. Droplet size, polydispersity and ζ-potential

The average droplet size of the nanoemulsions was determined by dynamic-light-scattering (DLS), using a Zetasizer Nano-ZS laser diffractometer (ATA Scientific, New South Wales, Australia), working at 633 nm and equipped with a backscatter detector (173°), which is used to specifically measure submicron particles. Polydispersity index (PdI), which represents the distribution of particle size, was also recorded from the instrument during the DLS measurement. PdI values near 1 indicate a heterogeneous or multimodal distribution of droplet sizes, whereas those near 0 give an idea of monomodal distribution (Gago et al., 2019). The electrophoretic mobility of oil droplets in nanoemulsions, also known as ζ-potential (mV) was determined by phase-analysis light scattering (PALS) using an automated capillary electrophoresis device (ATA Scientific, New South Wales, Australia), using a 633 nm laser at 25 °C. This determines the surface electrical charge of the droplets dispersed in the continuous phase. Samples were prior diluted in MiliQ water using a dilution ratio of 1:9 sample to water. Measurements of the parameters (droplet size polydispersity and ζ-potential) were performed over the following storage times - 0, 2, 4 and 6 months.

2.8.2. Antimicrobial activity

The antimicrobial activity of lemongrass and citral nanoemulsions were evaluated using inactivation of *B. cereus*. Three isolates of *B. cereus*

were used in this experiment: one reference isolate, (*B. cereus* ATCC 14579), one isolated from potato (*B. cereus* P4) and one isolated from milk (*B. cereus* M2). All isolates were provided by the Food Microbiology laboratory of Massey University. Briefly, *B. cereus* was cultured in Brain Heart Infusion (BHI) broth (Bacto™, Becton, Dickinson and Company, USA) at 30 °C for overnight. Once the bacterial numbers reached log 6 colony-forming units/mililiter (CFU/mL), 0.1 mL of the bacterial culture was transferred to a 1.5 mL Eppendorf tube with 0.1 mL of lemongrass or citral nanoemulsions. To confirm that the culture reached 6 log CFU/mL, optical density (OD) measurement was performed using spectrophotometry (Varioskan™ Lux, Thermo Fisher Scientific, Massachussetts, USA) at 600 nm. A correlation curve between OD600 and CFU/mL was established, allowing the estimation of bacterial concentration based on absorbance readings. The Eppendorf tube was incubated at 30 °C for 1 h, then centrifuged (Sigma® 6–16, John Morris Scientific Ltd., New Zealand) at 12 000×*g* for 5 min at 4 °C, the cell pellets after centrifugation were washed with sterile saline (0.85 %) via vortex (30 s) (Scilogex, Germany) and centrifugation (5000×*g* for 5 min). The concentrated cells were collected by removing saline and dissolving the final pellet in fresh sterile saline. Serial dilutions of the bacterial suspension were prepared and spread on Tryptic Soy Agar (TSA) (Difco™, Becton, Dickinson and Company, USA). The plates were incubated at 30 °C for 24 h. A control was performed with the same method by replacing the nanoemulsions with sterile saline water. The colonies on the plates were counted and CFU/mL were calculated using the formula of CFU/mL : CFU/ml = *(Number of colonies x dilution factor)/volume of culture plate.* The detection limit of the colonies on the plate is Log 1 CFU/mL. The antimicrobial activity determinations were performed just after preparation of the nanoemulsions and at each of the storage times (0, 2, 4 and 6 months).

2.8.3. Thermal stability

The stability of lemongrass and citral nanoemulsions were tested at different temperature and pHs according to the method described by Ramli et al., (2020), with slight modifications. For the thermal stability, nanoemulsions were exposed to the following temperatures: 30 °C, 60 °C and 90 °C for 15 min each. A heat block (Techne® Dri-Block® OB-3, Watson Victor LTD, New Zealand) was pre-set at different temperatures (30 °C, 60 °C and 90 °C) prior to each test. A thermometer was used to verify that the desired temperature was reached before treating the samples. After incubation, the samples were left at room temperature (21 ± 2 °C) to cool completely before testing for their antimicrobial properties by using MICs analysis. Untreated extract with pH 6 at room temperature (21 ± 2 °C) was used as a control.

2.9. Determination of minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) was performed in a 96-well microtiter plate using two-fold serial dilutions. Lemongrass or citral nanoemulsions with a concentration of 2 % was mixed and two-fold dilutions were prepared in Brain Heart Infusion (BHI) broth containing the inoculum. Column 12 was filled with the highest concentration of Lemongrass or citral nanoemulsions (1 %), while column 3 was filled with the lowest concentration of Lemongrass or citral nanoemulsions (0.02 %). Column 1 served as the negative control (no inoculum and antimicrobial agent) and column 2 served as the positive control (inoculum and antimicrobial agent). The MIC was determined as the lowest concentration of antimicrobial agent that inhibits microbial growth, and the absorbance was measured using a microtiter plate reader (Varioskan ™ LUX, UK) with reading <0.05 change in optical density $(OD)_{590}$ after incubated the sample for 24 h at 30 °C.

2.10. Statistical analysis

All experiments were performed in triplicate. Data were analyzed by one-way analysis of variance (ANOVA) (IBM SPSS version 29) and, where necessary, the Tukey test (α = 0.05) or *t*-test (α = 0.05). All results were expressed as mean ± standard deviation.

3. Results

3.1. Yield of lemongrass supercritical fluid extraction

Table 1 shows the yield collected from raw lemongrass at different pressure levels during supercritical fluid carbon dioxide extraction. The pressures are listed in bars and the yields are given as a percentage.

The results indicate a clear positive relationship between the applied pressure and the yield collected, highlighting the influence of pressure on the efficiency of the process. At the lowest pressure of 85 bar, the yield collected is minimal, at only 0.075 %. As the pressure increased to 100 bar, the yield more than tripled to 0.291 %. Further increases in pressure to 200 bar and 300 bar resulted in progressively higher yields of 0.428 % and 0.815 %, respectively.

3.2. Composition of active compounds by Gas Chromatography Mass Spectrometry (GCMS) analysis

The results of the Gas Chromatography Mass Spectrometry (GCMS) analysis of supercritical fluid extraction are presented in Table 2. Comparing chemical profile of lemongrass to its standard citral extract. The main bioactive components in lemongrass oil are neral and geranial, which together make up citral, a key compound valued for its fragrance and antimicrobial properties.

In the citral standard, neral and geranial were the primary components, at 44.08 % and 45.98 % respectively. In the SFE lemongrass extract, while neral and geranial were still dominant (25.25 % and 46.44 %, respectively), additional compounds were present, demonstrating a broader chemical profile. These included geraniol (4.18 %), caryophyllene (2.11 %), and junipercamphor (7.75 %), along with several other minor terpenes and sesquiterpenes, such as cis-α-bergamotene, germacrene D, and viridiflorol.

3.3. Antimicrobial activity

Table 3 shows the antimicrobial activity of lemongrass (LG) and citral (C) nanoemulsions at various concentrations (0.125 %, 0.250 %, 0.500 %, and 2.000 %) against three *B. cereus* isolates (ATCC 14579, P4, and M2) over a six-month storage period at 4 °C. Across all isolates, higher concentrations (0.500 % and 2.000 %) of both LG and C nanoemulsions consistently achieved the highest log reductions, indicating their ability to suppress bacterial growth. For instance, at 2 months, the 2.000 % LG concentration showed a 4.86 log reduction for *B. cereus* ATCC 14579 and effectively suppressed *B. cereus* P4 and *B. cereus* M2 to <1.00 CFU/mL. The effectiveness of the nanoemulsions decreased with storage time, particularly at lower concentrations. For example, for P4 with 0.125 % LG, the log reduction at 2 months was 3.66, but this dropped by 4 months. This suggests degradation or dissipation of active components over time, reducing antimicrobial efficacy.

The data (Table 3) highlights the concentration-dependent and time-dependent effects of lemongrass (LG) and citral (C) nanoemulsions on the growth of *B. cereus* isolates (ATCC 14579, P4, and M2). Across all isolates, higher concentrations of nanoemulsions (e.g., 2.000 %) consistently achieved the highest reductions in bacterial growth. For instance, at Day 0, both 0.500 % and 2.000 % LG or C nanoemulsions

Table 1
Yield collected of lemongrass at different pressures level.

Pressure (bar)	Yield collected (%)
85	0.075
100	0.291
200	0.428
300	0.815

Table 2
Composition of bioactive components in lemongrass and citral.

No.	Name of compound	Standard		Supercritical fluid extraction	
		Citral		Lemongrass	
		Retention time	Area (%)	Retention time	Area (%)
1	Neral	22.179	44.08	22.285	25.25
2	Geraniol	nd	nd	22.859	4.18
3	Geranial	23.561	45.98	23.740	46.44
4	Caryophyllene	nd	nd	30.497	2.11
5	Cis-.alpha.-Bergamotene	nd	nd	31.147	1.00
6	Geranial diethylacetal	nd	nd	31.262	0.75
7	Germacrene D	nd	nd	33.160	1.10
8	Bulnesene <alpha>	nd	nd	34.221	0.79
9	Muurolene <gamma>	nd	nd	34.557	0.81
10	Endo-1-bourbonanol	nd	nd	37.095	2.29
11	Junipercamphor	nd	nd	38.864	7.75
12	Viridiflorol	nd	nd	40.228	0.83
13	Intermedeol	nd	nd	40.444	1.42
14	Eudesm-7(11)-en-4-ol	nd	nd	41.871	1.29

*nd: not detected

achieved near-complete suppression of *B. cereus* P4 and *B. cereus* M2 (<1.00 log CFU/mL). In contrast, lower concentrations (0.125 % and 0.250 %) demonstrated more modest reductions initially and lost efficacy over time. For example, in *B. cereus* P4, 0.125 % LG reduced growth to 3.47 log CFU/mL at Day 0 but showed diminished effectiveness over time, with counts reaching 6.07 log CFU/mL at 6 months. This time-dependent decline suggests dissipation or degradation of the active components in the nanoemulsions.

Isolate-specific differences are also apparent, with ATCC 14579 showing the highest resistance to the treatments compared to *B. cereus* P4 and *B. cereus* M2. Even at 2.000 % LG, the growth of *B. cereus* ATCC 14579 remained at 2.06 log CFU/mL on Day 0, compared to <1.00 log CFU/mL for *B. cereus* P4 and *B. cereus* M2. Despite this resistance, *B. cereus* ATCC 14579 demonstrated relative stability in its response to the nanoemulsions over the six-month storage period, as no significant difference ($p > 0.05$) was observed in 2.000 % LG throughout the storage time. In contrast, significant differences ($p < 0.001$) were observed in *B. cereus* P4 and *B. cereus* M2.

3.4. Droplet size, polydispersity index and zeta potential

The physicochemical stability of lemongrass and citral nanoemulsions was assessed over six months of storage at 4 °C (Table 4), focusing on parameters such as droplet size, polydispersity index (PDI), and zeta potential.

Nanoemulsion stability is often evaluated through key physical characteristics such as droplet size, polydispersity index (PDI), and zeta potential. These parameters are critical in determining the stability, homogeneity, and functionality of nanoemulsions in various applications. Droplet size indicates the average diameter of the dispersed phase in the emulsion and affects stability, with smaller droplet sizes typically correlating with greater resistance to coalescence (McClements, 2012). The polydispersity index (PDI) reflects the size distribution of droplets within the emulsion, with values closer to 0 indicating a more uniform size distribution, essential for physical and chemical stability (Danaei et al., 2018). Zeta potential measures the surface charge of droplets and serves as an indicator of electrostatic repulsion between particles; higher absolute values signify greater colloidal stability by preventing aggregation (Honary & Zahir, 2013).

The data (Table 4) demonstrates significant changes ($p < 0.001$) in the physical stability of lemongrass nanoemmulsion (LG) over time, specifically in droplet size and zeta potential measurements. Similarly, for citral nanoemulsions (C), significant changes ($p < 0.001$) are

Table 3
Growth of *B. cereus* isolates (log CFU/ml), after exposure (60 min) to nanoemulsions supplemented with different concentrations of lemongrass (LG), citral (C), at day 0, and after 2, 4 and 6 months of storage at 4 °C.

B. cereus isolates	Concentration of nanoemulsions	Growth of *B. cereus* (log CFU/mL)			
		Day 0	2 months	4 months	6 months
ATCC 14579	Control	6.50 ± 0.71^{gB}	7.26 ± 0.25^{dC}	5.83 ± 0.60^{eA}	6.00 ± 0.00^{eAB}
	0.125 % LG	5.14 ± 0.15^{fB}	3.60 ± 0.43^{cA}	4.75 ± 0.21^{dB}	5.24 ± 0.34^{dB}
	0.250 % LG	3.19 ± 0.65^{deA}	2.63 ± 0.35^{bcA}	3.15 ± 0.21^{bA}	4.15 ± 0.21^{cB}
	0.500 % LG	3.20 ± 0.17^{cdB}	3.25 ± 0.03^{aBC}	2.36 ± 0.39^{aA}	3.50 ± 0.28^{bcC}
	2.000 % LG	2.06 ± 0.21^{aA}	2.40 ± 0.46^{aA}	2.35 ± 0.49^{aA}	2.50 ± 0.71^{aA}
	0.125 % C	3.77 ± 0.10^{eB}	4.30 ± 0.26^{bcA}	4.97 ± 0.06^{dC}	4.97 ± 0.06^{dC}
	0.250 % C	2.23 ± 0.40^{abA}	3.23 ± 0.20^{aB}	4.00 ± 0.00^{cC}	4.00 ± 0.00^{cC}
	0.500 % C	2.84 ± 0.15^{bcdB}	2.24 ± 0.34^{abA}	3.15 ± 1.62^{bB}	3.00 ± 0.00^{abB}
	2.000 % C	2.43 ± 0.23^{abA}	2.80 ± 0.14^{abB}	3.15 ± 0.21^{bC}	3.00 ± 0.00^{abBC}
P4	Control	7.10 ± 0.17^{dB}	7.30 ± 0.00^{dB}	6.35 ± 0.49^{cA}	6.39 ± 0.36^{cA}
	0.125 % LG	3.47 ± 0.67^{bA}	4.54 ± 0.09^{cB}	6.07 ± 0.40^{cC}	6.07 ± 0.68^{bcC}
	0.250 % LG	3.47 ± 0.00^{bA}	3.60 ± 0.43^{abA}	5.30 ± 0.43^{bB}	5.24 ± 0.34^{bB}
	0.500 % LG	$<1.00 \pm 0.00^{aA}$	$<1.00 \pm 0.00^{aaA}$	4.92 ± 0.11^{abB}	5.24 ± 0.34^{bB}
	2.000 % LG	$<1.00 \pm 0.00^{aA}$	$<1.00 \pm 0.00^{acA}$	4.49 ± 0.03^{aC}	4.00 ± 0.00^{aB}
	0.125 % C	4.30 ± 0.43^{cA}	4.24 ± 0.34^{acA}	6.79 ± 0.61^{cC}	5.65 ± 0.91^{bcB}
	0.250 % C	3.33 ± 0.20^{bA}	3.30 ± 0.26^{aA}	6.25 ± 0.15^{cC}	5.31 ± 0.58^{bcB}
	0.500 % C	$<1.00 \pm 0.00^{aA}$	$<1.00 \pm 0.00^{aA}$	4.86 ± 0.27^{abC}	4.15 ± 0.21^{aB}
	2.000 % C	$<1.00 \pm 0.00^{aA}$	$<1.00 \pm 0.00^{aA}$	4.52 ± 0.16^{abC}	3.69 ± 0.53^{aB}
M2	Control	6.59 ± 0.25^{eAB}	7.03 ± 0.05^{be}	6.67 ± 0.58^{dAB}	6.26 ± 0.24^{fA}
	0.125 % LG	3.00 ± 0.00^{cA}	4.15 ± 0.21^{dB}	6.46 ± 0.27^{cdC}	6.46 ± 0.15^{fC}
	0.250 % LG	3.44 ± 0.05^{dB}	2.74 ± 0.37^{bA}	6.25 ± 0.22^{cdC}	5.98 ± 0.03^{eC}
	0.500 % LG	1.00 ± 0.00^{aA}	$<1.00 \pm 0.00^{aA}$	4.82 ± 0.31^{abB}	4.63 ± 0.21^{cB}
	2.000 % LG	$<1.00 \pm 0.00^{aA}$	$<1.00 \pm 0.00^{aA}$	4.18 ± 0.24^{aC}	2.50 ± 0.28^{bB}
	0.125 % C	3.23 ± 0.34^{cdA}	4.26 ± 0.24^{dB}	7.13 ± 0.38^{dD}	5.50 ± 0.71^{deC}
	0.250 % C	2.54 ± 0.09^{bA}	3.70 ± 0.12^{cB}	6.39 ± 0.35^{cdD}	5.48 ± 0.43^{deC}
	0.500 % C	$<1.00 \pm 0.00^{aA}$	$<1.00 \pm 0.00^{aA}$	5.10 ± 0.17^{bcB}	5.15 ± 0.21^{cdB}
	2.000 % C	$<1.00 \pm 0.00^{aA}$	$<1.00 \pm 0.00^{aA}$	4.37 ± 0.19^{aC}	1.39 ± 0.12^{aB}

LG: lemongrass; C: citral.
The values followed by the same lowercase, in the same column and by the same uppercase in the same row are not significantly different by Tukey test, at $P < 0.05$.

Table 4
Physicochemical stability parameters of lemongrass and citral nanoemulsions at 2.000 % concentration over 6 months of storage at 4 °C.

Duration	0 Days		2 Months		4 Months		6 Months	
Nanoemulsion	LG	C	LG	C	LG	C	LG	C
Droplet size (nm)	86.32 ± ^{A} 0.66	32.86 ± ^{A} 1.37	346.03 ± ^{B} 21.98	161.60 ± ^{AB} 43.69	516.63 ± ^{C} 20.24	281.65 ± ^{BC} 81.53	494.47 ± ^{C} 8.88	350.27 ± ^{C} 85.93
Polydispersity index	0.50 ± ^{A} 0.00	0.85 ± ^{B} 0.04	0.89 ± ^{A} 0.14	0.54 ± ^{A} 0.18	0.85 ± ^{A} 0.07	0.33 ± ^{A} 0.03	0.81 ± ^{A} 0.27	0.89 ± ^{B} 0.13
Zeta potential (mV)	−44.01 ± ^{C} 1.69	−31.08 ± ^{A} 0.45	−42.26 ± ^{C} 0.86	−36.83 ± ^{AB} 3.25	−38.94 ± ^{B} 1.18	−38.35 ± ^{B} 0.96	−33.63 ± ^{A} 1.45	−32.88 ± ^{AB} 2.57

LG: lemongrass; C: citral.
The values followed by the same uppercase letter in the same row are not significantly different by Tukey test, at $P < 0.05$.

observed in droplet size and the polydispersity index (PDI). Initially, at Day 0, LG nanoemulsions showed smaller droplet sizes (86.32 ± 0.66 nm) compared to C nanoemulsions (32.86 ± 1.37 nm). However, over time, both formulations showed an increase in droplet size, with LG nanoemulsions reaching 494.47 ± 8.88 nm and C nanoemulsions increasing to 350.27 ± 85.93 nm by 6 months. This growth in droplet size suggests coalescence or aggregation of the droplets, which could compromise the stability of the nanoemulsions. Notably, LG nanoemulsions consistently showed larger droplet sizes than C nanoemulsions at all time points, indicating potential differences in their structural stability under similar storage conditions.

Fig. 1 illustrates the relationship between droplet size and the antimicrobial activity of lemongrass nanoemulsion against *Bacillus cereus* isolate P4. Initially, the droplet size is small (~100 nm) but increases steadily over time, reaching approximately 500 nm by 6 months. The antimicrobial activity, represented by the microbial count (log CFU/mL), remains low (<1.0 log CFU/mL) during the first 2 months. However, at 4 months, there is a sharp increase in the microbial count (~4.0 log CFU/mL), indicating a weakening of antimicrobial activity. A similar trend is observed at 6 months, further confirming a decline in antimicrobial effectiveness over time. This chart suggests a possible correlation between droplet size and antimicrobial activity, with reduced effectiveness occurring as the droplet size reaches its maximum.

The PDI values also reveal insights into the uniformity of the nanoemulsion formulations. For LG, the PDI increased from 0.50 ± 0.00 on Day 0–0.81 ± 0.27 at 6 months, indicating a broader size distribution and reduced stability over time. In contrast, C nanoemulsions showed fluctuating PDI values, with an initial high value of 0.85 ± 0.04 at Day 0, decreasing to 0.33 ± 0.03 at 4 months before rising again to 0.89 ± 0.13 at 6 months. These fluctuations suggest that citral nanoemulsions experience temporary stabilization but ultimately face instability like lemongrass nanoemulsions.

The zeta potential values, which indicate the surface charge and colloidal stability, decreased over time for both LG and C nanoemulsions. Initially, LG nanoemulsions had a zeta potential of −44.01 ± 1.69 mV, which increased to −33.63 ± 1.45 mV at 6 months. Similarly, C nanoemulsions started at −31.08 ± 0.45 mV and decreased to −32.88 ± 2.57 mV by 6 months. While the negative charge remains sufficient to impart some stability, the reduction in zeta potential suggests a decline in repulsive forces, potentially contributing to aggregation and droplet growth.

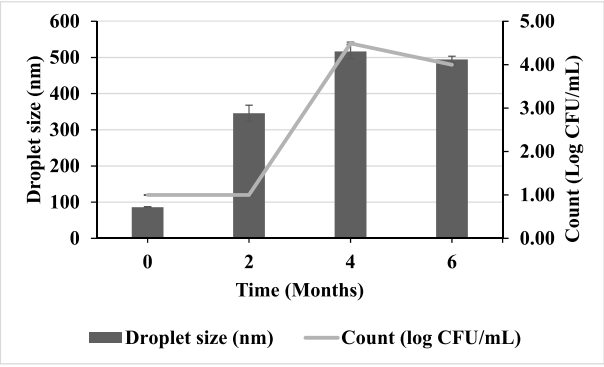

Fig. 1. Correlation between droplet size and antimicrobial activity of lemongrass nanoemulsion against *B. cereus* isolate P4.

3.5. Thermal stability

The minimum inhibition concentation (MIC) of citral and lemongrass nanoemulsions remained constant at 0.125 % across all tested temperatures (21.0 °C, 30.0 °C, 60.0 °C, and 90.0 °C) for the three *B. cereus* isolates: ATCC 14579, P4, and M2. This constancy indicates that the formulations are thermally stable and do not exhibit degradation or concentration loss when subjected to increasing temperatures, up to 90 °C.

4. Discussion

Supercritical Fluid Extraction (SFE) is a highly efficient method for isolating bioactive compounds from natural sources like lemongrass, utilizing the unique properties of supercritical carbon dioxide to achieve selective extraction while preserving the integrity of sensitive bioactives. Under optimized conditions, SFE at 300 bar achieved a peak yield of 0.815 %, illustrating its capability to maximize extraction efficiency. These results are consistent with Rodrigues et al., (2018), who observed enhanced yields for eucalyptus leaves under similar pressure conditions when combined with co-solvents. However, as Pokrovskiy et al., (2019) noted, the relationship between pressure and yield is not universally linear; certain matrices, such as black coffee oil and cannabis, exhibit reduced yields at elevated pressures due to plant-specific matrix composition and saturation effects.

While high pressures offer substantial yields, 85 bar was identified as the optimal extraction pressure for lemongrass due to its practical and functional advantages. Extracts obtained at this moderate pressure retained a liquid state, avoiding the waxy, semi-solid phase that complicates solubility at higher pressures. Furthermore, from the preliminary study, lemongrass extracts obtained at 85 bar exhibited superior antimicrobial activity against Gram-positive and Gram-negative bacteria (Supplementary 1). These findings align with Calva-Cruz et al., (2021), who demonstrated that optimized SFE conditions for *Lippia graveolens* produced antimicrobial compounds effective against multidrug-resistant pathogens, *Enterococcus faecalis* and *S. aureus* isolates even under conditions that were less than those required for maximum yield.

Nanoemulsions formulated with lemongrass and citral in the present study consistently demonstrated strong antimicrobial potential, largely attributed to their small initial droplet sizes, often below 200 nm, which enhance stability and microbial interaction. Both lemongrass and citral nanoemulsions exhibited comparable antimicrobial activity, suggesting that citral, the major active compound in lemongrass oil, is primarily responsible for the observed efficacy. Moreover, findings from our preliminary study (Supplementary 2) revealed that lemongrass nanoemulsions exhibited significantly higher antimicrobial activity

compared to lemongrass essential oil, achieving comparable effects at five times lower concentrations. For instance, lemongrass and citral nanoemulsions reported in this study have particle sizes ranging from 32 to 86 nm, which gradually increased over time due to coalescence and aggregation during storage (Fig. 1). Citronella oil nanoemulsions exhibited optimized particle sizes around 79 nm, with minimal changes over 28 days under low-temperature storage, underscoring the importance of controlled environments for stability (Somala et al., 2022). Notably, smaller droplet sizes, particularly those below 100 nm, are associated with enhanced antimicrobial efficacy due to improved interactions with microbial membranes (Sedaghat Doost et al., 2020).

Zeta potential, a measure of electrostatic stability, initially exceeded −30 mV in most nanoemulsions, indicating sufficient colloidal stability. However, over six months, lemongrass nanoemulsions showed a decline in zeta potential from −44 mV to −33 mV, reflecting reduced electrostatic repulsion and a heightened risk of droplet aggregation. These trends are consistent with findings by Carvalho et al., (2018), who emphasized the critical role of maintaining a zeta potential above −30 mV to prevent destabilization. Stability-enhancing agents, such as polymer encapsulation or synergistic surfactants, have been shown to mitigate these declines, maintaining stability under various storage conditions.

The antimicrobial activity of lemongrass nanoemulsions was particularly prominent during the first four months of storage, achieving significant log reductions against *B. cereus* ATCC 14579 and complete suppression of *B. cereus* P4 and *B. cereus* M2 isolates at higher concentrations (2.000 %). This efficacy aligns with studies by Garcia et al., (2022) and do Carmo Silva et al., (2020) which attribute such performance to enhanced solubility, bioavailability, and microbial membrane interactions facilitated by nanoemulsion formulation. However, in the present study, degradation of antimicrobial activity was observed that started at four months and was associated with droplet coalescence and declining zeta potential. These challenges underscore the need for advanced stabilization strategies, such as polymer encapsulation (Maurya et al., 2021) or controlled-release systems (Jamali et al., 2021), to extend functional efficacy. Studies by Gago et al., (2019) highlighted the critical role of optimal storage conditions in maintaining nanoemulsion stability. Specifically, the highest concentration of lemongrass nanoemulsion (2.5 %) retained its antimicrobial efficacy against *E. coli* for up to six months when stored at 1 °C, compared to significantly reduced stability at 25 °C.

When comparing stability across time, lemongrass nanoemulsions demonstrated relatively prolonged stability compared to other essential oils. For example, citronella oil nanoemulsions exhibited degradation in particle size and antimicrobial activity within 28 days (Touayar et al., 2023), and thymol nanoemulsions showed a decline in efficacy after two months due to electrostatic instability (Kumari et al., 2018). In contrast, for this study, lemongrass nanoemulsion maintained stability for up to four months under optimized storage conditions, with particle sizes increasing from an initial 86.32 ± 0.66 nm to over 350 nm. Notably, both nanoemulsions lemongrass and citral displayed comparable efficacy and stability over time, underscoring their potential as robust antimicrobial agents with consistent performance during extended storage. This highlights the importance of optimal storage conditions to maintain their functionality and efficacy.

Thermal stability testing has reinforced the robustness of lemongrass and citral nanoemulsions across a broad temperature range (21 °C–90 °C), making them suitable for heat-intensive applications in food manufacture. Singh et al., (2020) similarly reported that surfactant-stabilized nanoemulsions effectively resist thermal degradation, maintaining structural integrity and efficacy under industrial processing conditions. Consistent efficacy across *B. cereus* ATCC 14579, *B. cereus* P4, and *B. cereus* M2 underscores their broad-spectrum applicability. Research conducted by Borba et al., (2019) demonstrated that all nanoemulsions showed exceptional resistance to droplet coalescence during thermal treatments and under various storage conditions, exhibiting low Ostwald ripening rates. However, the retention of β-carotene within the nanoemulsions depended on storage temperature, the exclusion of light, and the encapsulation efficiency (%EE). Studies by da Silva Gundel et al., (2018) further emphasized the importance of thermal stability in preserving antimicrobial properties during extended use.

Despite their promising performance, isolate-specific differences in susceptibility present challenges. For instance, *B. cereus* ATCC 14579 demonstrated greater resistance compared to *B. cereus* P4 and *B. cereus* M2, highlighting the necessity of tailoring nanoemulsion formulations to target diverse pathogens. Similar variability has been reported by do Carmo Silva et al., (2020) in the susceptibility of *L. monocytogenes* isolates, attributed to genetic and structural differences among microbial species.

The variability in efficacy of nanoemulsions against different isolates of *Bacillus cereus* highlights isolate-dependent susceptibility, influenced by genetic, structural, and physiological differences among isolates. Resistant isolates, such as ATCC 14579, demonstrate higher tolerance compared to more susceptible isolates like P4 and M2. This resistance may come from unique genetic and structural characteristics, including differences in cell wall composition, charge, and permeability, which alter their interaction with nanoemulsion components like citral or lemongrass oil (Ehling-Schulz et al., 2019). Moreover, resistant isolate like ATCC 14579 may possess more effective repair mechanisms for cell membrane damage, enabling them to maintain viability over extended treatment periods, whereas susceptible isolates experience a decline in efficacy over time.

Genomic studies further emphasize this variability, showing distinct phylogenetic groupings and differences in virulence-associated genes among isolates (Didouh et al., 2023). This genomic diversity directly impacts susceptibility to antimicrobials and other interventions, as evidenced by biofilm composition differences, such as extracellular DNA (eDNA) content, which can influence resistance to enzymatic degradation and antimicrobial agents (Lim et al., 2021). Ultimately, the interaction between nanoemulsions and *B. cereus* isolates is a complex interplay of isolate-specific traits, emphasizing the importance of understanding variability in pathogen behavior to develop effective and consistent antimicrobial strategies.

5. Conclusion

This study demonstrates that supercritical fluid-extracted lemongrass and citral nanoemulsions exhibit strong antimicrobial activity and stability, particularly under optimized storage and thermal conditions. Despite slight variations in droplet size and zeta potential, both nanoemulsions proved effective for controlling *B. cereus*.

Notably, the highest yield does not equate to maximum antimicrobial efficiency. An optimized extraction pressure of 85 bar at 40 °C provided the lowest minimum inhibitory concentration (MIC) activity, highlighting the importance of tailored extraction conditions.

Throughout storage, higher concentrations of nanoemulsions (0.500 % and 2.000 %) consistently achieved the highest log reductions, effectively suppressing bacterial growth. Both nanoemulsions remained efficient for up to four months, but their stability declined thereafter, as indicated by increased droplet size, polydispersity index, reduced zeta potential, and increased viability of *B. cereus*.

Both formulations exhibited similar antimicrobial behavior, despite isolate-dependent variations among *B. cereus*. Moreover, the nanoemulsions demonstrated thermal stability, maintaining efficacy even under high-temperature exposure, underscoring their robustness in heat-intensive applications.

To enhance the functionality and expand the applications of lemongrass and citral nanoemulsions, advanced stabilization techniques, such as polymer encapsulation or synergistic emulsifiers, are recommended. Future research should also explore their impact on sensory properties, including aroma, taste, and texture, to ensure

consumer acceptance in food products. Investigating these aspects could significantly extend their shelf life and utility in industrial, clinical, and food preservation.

CRediT authorship contribution statement

Ili Syuhada Mohd Daud: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nor Khaizura Mahmud Ab Rashid:** Writing – review & editing, Supervision, Resources, Conceptualization. **Jon Palmer:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Steve Flint:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.106526.

Data availability

Data will be made available on request.

References

Barradas, T. N., & de Holanda e Silva, K. G. (2020). Nanoemulsions of essential oils to improve solubility, stability and permeability: A review. *Environmental Chemistry Letters, 19*(2), 1153–1171. https://doi.org/10.1007/s10311-020-01142-2

Borba, C. M., Tavares, M. N., Macedo, L. P., Araujo, G. S., Furlong, E. B., Dora, C. L., & Burkert, J. F. M. (2019). Physical and chemical stability of beta-carotene nanoemulsions during storage and thermal process. *Food Research International, 121*, 229–237. https://doi.org/10.1016/j.foodres.2019.03.045

Calva-Cruz, O. J., Badillo-Larios, N. S., De Leon-Rodriguez, A., Espitia-Rangel, E., Gonzalez-Garcia, R., Turrubiartes-Martinez, E. A., Castro-Gallardo, A., & Barba de la Rosa, A. P. (2021). *Lippia graveolens* HBK oleoresins, extracted by supercritical fluids, showed bactericidal activity against multidrug resistance *Enterococcus faecalis* and Staphylococcus aureus strains. *Drug Development and Industrial Pharmacy, 47*(10), 1546–1555. https://doi.org/10.1080/03639045.2021.2008417

Carvalho, P. M., Felicio, M. R., Santos, N. C., Goncalves, S., & Domingues, M. M. (2018). Application of light scattering techniques to nanoparticle characterization and development. *Front Chem, 6*, 237. https://doi.org/10.3389/fchem.2018.00237

da Silva Gundel, S., de Souza, M. E., Quatrin, P. M., Klein, B., Wagner, R., Gundel, A., Vaucher, R. A., Santos, R. C. V., & Ourique, A. F. (2018). Nanoemulsions containing *Cymbopogon flexuosus* essential oil: Development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. *Microbial Pathogenesis, 118*, 268–276. https://doi.org/10.1016/j.micpath.2018.03.043

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics, 10*(2). https://doi.org/10.3390/pharmaceutics10020057

Didouh, N., Achek, R., Celandroni, F., Ghelardi, E., Mazzantini, D., Postollec, F., Moussa-Boudjemaa, B., Ouoba, L. I. I., Tomaso, H., Linde, J., El-Adawy, H., & Patarata, L. (2023). Genomic diversity and virulence genes characterization of *Bacillus cereus* sensu lato isolated from processing equipment of an Algerian dairy plant. *Journal of Food Quality, 2023*, 1–11. https://doi.org/10.1155/2023/5703334

do Carmo Silva, L., Miranda, M., de Freitas, J. V., Ferreira, S. F. A., de Oliveira Lima, E. C., de Oliveira, C. M. A., Kato, L., Terezan, A. P., Rodriguez, A. F. R., Faria, F., de Almeida Soares, C. M., & Pereira, M. (2020). Antifungal activity of *Copaiba* resin oil in solution and nanoemulsion against *Paracoccidioides* spp. *Brazilian Journal of Microbiology, 51*(1), 125–134. https://doi.org/10.1007/s42770-019-00201-3

Ehling-Schulz, M., Lereclus, D., & Koehler, T. M. (2019). The *Bacillus cereus* group: Bacillus species with pathogenic potential. *Microbiology Spectrum, 7*(3). https://doi.org/10.1128/microbiolspec.GPP3-0032-2018

Faheem, F., Liu, Z. W., Rabail, R., Haq, I. U., Gul, M., Bryla, M., Roszko, M., Kieliszek, M., Din, A., & Aadil, R. M. (2022). Uncovering the industrial potentials of lemongrass essential oil as a food preservative: A review. *Antioxidants, 11*(4). https://doi.org/10.3390/antiox11040720

Fei, P., Xu, Y., Zhao, S., Gong, S., & Guo, L. (2019). Olive oil polyphenol extract inhibits vegetative cells of *Bacillus cereus* isolated from raw milk. *Journal of Dairy Science, 102*(5), 3894–3902. https://doi.org/10.3168/jds.2018-15184

Gago, C. M. L., Artiga-Artigas, M., Antunes, M. D. C., Faleiro, M. L., Miguel, M. G., & Martin-Belloso, O. (2019). Effectiveness of nanoemulsions of clove and lemongrass essential oils and their major components against *Escherichia coli* and *Botrytis cinerea*. *J Food Sci Technol, 56*(5), 2721–2736. https://doi.org/10.1007/s13197-019-03762-1

Gao, S., Liu, G., Li, J., Chen, J., Li, L., Li, Z., Zhang, X., Zhang, S., Thorne, R. F., & Zhang, S. (2020). Antimicrobial activity of lemongrass essential oil (cymbopogon flexuosus) and its active component citral against dual-species biofilms of *Staphylococcus aureus* and *Candida* species. *Frontiers in Cellular and Infection Microbiology, 10*, Article 603858. https://doi.org/10.3389/fcimb.2020.603858

Garcia, C. R., Malik, M. H., Biswas, S., Tam, V. H., Rumbaugh, K. P., Li, W., & Liu, X. (2022). Nanoemulsion delivery systems for enhanced efficacy of antimicrobials and essential oils. *Biomaterials Science, 10*(3), 633–653. https://doi.org/10.1039/d1bm01537k

Guo, H., Yu, P., Yu, S., Wang, J., Zhang, J., Zhang, Y., Liao, X., Wu, S., Ye, Q., Yang, X., Lei, T., Zeng, H., Pang, R., Zhang, J., Wu, Q., & Ding, Y. (2021). Incidence, toxin gene profiling, antimicrobial susceptibility, and genetic diversity of *Bacillus cereus* isolated from quick-frozen food in China. *Lwt, 140*. https://doi.org/10.1016/j.lwt.2020.110824

Herzyk, F., Pilakowska-Pietras, D., & Korzeniowska, M. (2024). Supercritical extraction techniques for obtaining biologically active substances from a variety of plant byproducts. *Foods, 13*(11). https://doi.org/10.3390/foods13111713

Honary, S., & Zahir, F. (2013). Effect of zeta potential on the properties of nano-drug delivery systems - a review (Part 1). *Tropical Journal of Pharmaceutical Research, 12*(2). https://doi.org/10.4314/tjpr.v12i2.19

Jamali, S. N., Assadpour, E., Feng, J., & Jafari, S. M. (2021). Natural antimicrobial-loaded nanoemulsions for the control of food spoilage/pathogenic microorganisms. *Advances in Colloid and Interface Science, 295*, Article 102504. https://doi.org/10.1016/j.cis.2021.102504

Kumari, S., Kumaraswamy, R. V., Choudhary, R. C., Sharma, S. S., Pal, A., Raliya, R., Biswas, P., & Saharan, V. (2018). Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean. *Scientific Reports, 8*(1), 6650. https://doi.org/10.1038/s41598-018-24871-5

Lim, E. S., Baek, S. Y., Oh, T., Koo, M., Lee, J. Y., Kim, H. J., & Kim, J. S. (2021). Strain variation in Bacillus cereus biofilms and their susceptibility to extracellular matrix-degrading enzymes. *PLoS One, 16*(6), Article e0245708. https://doi.org/10.1371/journal.pone.0245708

Liu, Q., Huang, H., Chen, H., Lin, J., & Wang, Q. (2019). Food-grade nanoemulsions: Preparation, stability and application in encapsulation of bioactive compounds. *Molecules, 24*(23). https://doi.org/10.3390/molecules24234242

Maurya, A., Singh, V. K., Das, S., Prasad, J., Kedia, A., Upadhyay, N., Dubey, N. K., & Dwivedy, A. K. (2021). 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, 12*, Article 751062. https://doi.org/10.3389/fmicb.2021.751062

McClements, D. J. (2012). Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter, 8*(6), 1719–1729. https://doi.org/10.1039/c2sm06903b

McGaw, D. R., Clarke, W., & Maharaj, S. (2016). Comparison of supercritical fluid extraction with steam distillation for the extraction of bay oil from bay (*Pimenta Racemosa*) leaves. *International Journal of Engineering Science Invention, 5*(1), 51–55.

Moreira, S. A., Alexandre, E. M. C., Pintado, M., & Saraiva, J. A. (2019). Effect of emergent non-thermal extraction technologies on bioactive individual compounds profile from different plant materials. *Food Research International, 115*, 177–190. https://doi.org/10.1016/j.foodres.2018.08.046

Mushtaq, A., Mohd Wani, S., Malik, A. R., Gull, A., Ramniwas, S., Ahmad Nayik, G., Ercisli, S., Alina Marc, R., Ullah, R., & Bari, A. (2023). Recent insights into Nanoemulsions: Their preparation, properties and applications. *Food Chemistry X, 18*, Article 100684. https://doi.org/10.1016/j.fochx.2023.100684

Nejia, H., Severine, C., Jalloul, B., Mehrez, R., & Stephane, C. J. (2013). Extraction of essential oil from *Cupressus sempervirens*: Comparison of global yields, chemical composition and antioxidant activity obtained by hydrodistillation and supercritical extraction. *Natural Product Research, 27*(19), 1795–1799. https://doi.org/10.1080/14786419.2012.755680

Pokrovskiy, O. I., Prokopchuk, D. I., Kostenko, M. O., Ustinovich, K. B., Parenago, O. O., & Lunin, V. V. (2019). Effect of pressure on the efficiency of supercritical fluid extraction of black coffee oil. *Russian Journal of Physical Chemistry B, 12*(7), 1176–1181. https://doi.org/10.1134/s1990793118070096

Radmehr, B., Zaferanloo, B., Tran, T., Beale, D. J., & Palombo, E. A. (2020). Prevalence and characteristics of *Bacillus cereus* group isolated from raw and pasteurised milk. *Current Microbiology, 77*(10), 3065–3075. https://doi.org/10.1007/s00284-020-02129-6

Ramli, S., Rukayadi, Y., Shaari, K., & Radu, S. (2020). Toxicity analysis of *syzygium polyanthum* (wight) walp. Leaves extract and its stability against different pH and temperature. *Emirates Journal of Food and Agriculture*. https://doi.org/10.9755/ejfa.2020.v32.i6.2116

Rodrigues, V. H., de Melo, M. M. R., Portugal, I., & Silva, C. M. (2018). Supercritical fluid extraction of Eucalyptus globulus leaves. Experimental and modelling studies of the

influence of operating conditions and biomass pretreatment upon yields and kinetics. *Separation and Purification Technology, 191*, 173–181. https://doi.org/10.1016/j.seppur.2017.09.026

Sedaghat Doost, A., Nikbakht Nasrabadi, M., Kassozi, V., Nakisozi, H., & Van der Meeren, P. (2020). Recent advances in food colloidal delivery systems for essential oils and their main components. *Trends in Food Science & Technology, 99*, 474–486. https://doi.org/10.1016/j.tifs.2020.03.037

Somala, N., Laosinwattana, C., & Teerarak, M. (2022). Formulation process, physical stability and herbicidal activities of *Cymbopogon nardus* essential oil-based nanoemulsion. *Scientific Reports, 12*(1), Article 10280. https://doi.org/10.1038/s41598-022-14591-2

Touayar, M., Zayani, R., Messaoud, C., & Salman, H. (2023). Influence of droplet size on the antibacterial efficacy of citral and citronella oil nanoemulsions in polysaccharide coated fresh-cut apples. *Scientific Reports, 13*(1), Article 10460. https://doi.org/10.1038/s41598-023-37528-9

Yang, S., Wang, Y., Ren, F., Wang, X., Zhang, W., Pei, X., & Dong, Q. (2023). The sources of *Bacillus cereus* contamination and their association with cereulide production in dairy and cooked rice processing lines. *Food Quality and Safety, 7*. https://doi.org/10.1093/fqsafe/fyad023

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine, 13*, 20. https://doi.org/10.1186/s13020-018-0177-x