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From extraction to application: Nanoemulsified lemongrass oil for biofilm and spore control in food preservation

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ABSTRACT

Bacillus cereus is a spore-forming, toxin-producing pathogen that poses a persistent threat to global food safety due to its resistance to heat, disinfectants, and its ability to form biofilms. This review highlights the antimicrobial potential of lemongrass essential oil (LEO) and its major compound, citral, from traditional use to its modern application through nanoemulsion systems. It critically examines how extraction methods affect citral content and bioactivity, and how nanoemulsification enhances LEO's stability, solubility, and efficacy against *B. cereus* spores and biofilms. Applications include dairy, meat, and fresh produce preservation, where LEO-based coatings, packaging, and sanitizers offer clean-label alternatives to synthetic preservatives. The review also explores regulatory and safety concerns and identifies gaps in sensory effects, long-term stability, and dosing optimization. Overall, citral-rich LEO nanoemulsions represent a promising, sustainable strategy to improve microbial safety and shelf life in food systems affected by *B. cereus*.

1. Overview of food safety and the need for natural antimicrobials

1.1. *Common foodborne pathogens with a focus on Bacillus cereus*

Food safety remains a critical global issue, with the World Health Organization (WHO) estimating that contaminated food causes approximately 600 million cases of foodborne illnesses and 420,000 deaths annually, with children under five accounting for nearly 40% of the disease burden (WHO, 2024). The challenges associated with food safety arise from multiple factors, including microbial contamination, chemical residues, and physical hazards, all of which compromise food quality and consumer health. Biofilms formed by foodborne pathogens such as *Listeria monocytogenes* on food contact surfaces act as persistent reservoirs of contamination, making them difficult to eradicate with conventional sanitisation methods (Mazaheri et al., 2021). These biofilms also contribute to antimicrobial resistance (AMR), further increasing the incidence and severity of foodborne outbreaks (Pai et al., 2023). With rapid population growth, urbanisation, and globalised food supply chains, ensuring access to safe and nutritious food has become increasingly challenging. Shifts in food production systems and dietary preferences toward minimally processed and ready-to-eat foods increase contamination risks (Bhatia et al., 2024).

Furthermore, emerging and re-emerging pathogens such as *Salmonella*, *Listeria monocytogenes*, pathogenic *Escherichia coli* strains, including Shiga toxin–producing (EHEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC) types and *Campylobacter* are evolving to exploit new ecological niches in food matrices (Newell et al., 2010). Climate change adds another layer of complexity by altering agricultural systems, increasing the presence of disease carriers, and enhancing the survival and transmission of foodborne pathogens across global supply chains (Bongben, 2024). Microbial contamination remains the most common cause of foodborne illnesses globally, contributing to significant public health and economic burden. Outbreaks frequently originate from contaminated fresh produce, dairy, and meat products, with pathogens such as Norovirus, *Salmonella*, and *B. cereus* among the leading culprits (Warmate and Onarinde, 2023). In the United States alone, the annual economic burden of foodborne illnesses is estimated at over USD 15 billion, accounting for medical costs, productivity losses, and legal liabilities (CDC, 2024). In regions such as Southeast Asia, sub-Saharan Africa, and countries like Nepal, similar patterns are observed, particularly in low and middle-income settings, where inadequate sanitation, informal food markets, and weak regulatory oversight heighten food safety risks (Subedi et al., 2024; Wallace et al., 2022).

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A key mechanism of microbial contamination is cross-contamination. The transfer of pathogens from raw ingredients, utensils, or contaminated surfaces to ready-to-eat foods during processing or handling (Pakdel et al., 2023). This is especially problematic in facilities with poor hygiene practices or inadequate equipment maintenance. For example, *L. monocytogenes* biofilms on stainless steel surfaces in processing plants have been shown to resist standard cleaning agents, allowing persistent contamination across production cycles (Lake et al., 2024). Likewise, fresh produce is frequently contaminated using irrigation water polluted with faecal matter, a common route for *E. coli* and *Salmonella* contamination (Gurtler and Gibson, 2022).

A key pathogen of interest is *B. cereus*, which is capable of withstanding heat treatments and surviving in adverse environmental conditions due to its spore-forming ability. These spores can survive typical cooking temperatures and when cooked foods such as rice or pasta are held at improper storage temperatures, germinate into vegetative cells that produce enterotoxins responsible for vomiting or diarrhoeal syndromes. A notable case occurred in 2021 in China, where rice noodles contaminated with *B. cereus* led to 198 reported cases of foodborne illness (Li et al., 2023). These cases highlight the need to enhance food safety systems through advanced detection methods, strict hygiene measures, and coordinated efforts across sectors.

Importantly, there is growing interest in natural antimicrobial solutions, including plant-derived compounds like essential oils, as sustainable alternatives to chemical preservatives and conventional sanitisation agents. These innovations may offer safer, more consumer-acceptable strategies for controlling microbial contamination and addressing antimicrobial resistance (Karnwal and Malik, 2024). Table 1 below summarises notable foodborne illness outbreaks from 2017 to 2024, illustrating the diversity of pathogens, contamination sources, and public health impacts across multiple countries.

1.2. Pathogenic mechanisms of B. cereus

1.2.1. Biofilm formation and food industry implications

B. cereus is a Gram-positive, spore-forming bacterium commonly associated with foodborne illnesses due to its ability to persist in diverse environments. Its growth is influenced by temperature and pH, with most strains thriving between 30 and 40 °C, though psychotropic strains can grow at temperatures as low as 4 °C, posing a risk to refrigerated foods (Rodrigo et al., 2021). The optimum pH range for growth is between 5.0 and 8.0, while acidic conditions (pH <5) significantly reduce proliferation and toxin production (Wang et al., 2024a). Compared with non-spore-forming foodborne pathogens such as *E. coli* and *Salmonella* spp., *B. cereus* presents a unique control challenge due to its ability to simultaneously form spores and biofilms, enabling survival through thermal processing, sanitation, and cold storage.

One of the critical survival mechanisms of *B. cereus* in food environments is its ability to form biofilms. These structured microbial communities are enclosed in a self-produced extracellular matrix that adheres tightly to surfaces such as stainless steel and plastic. Biofilms not only enable persistence under adverse conditions but also significantly reduce the efficacy of cleaning and sanitization protocols (Ghosh et al., 2024). Strain-dependent variability in biofilm architecture and extracellular polymeric substances (EPS) composition influences resilience. For instance, strain ATCC 10987 exhibits unique responses to matrix-degrading enzymes, indicating the complexity and heterogeneity of biofilm structures (Lim et al., 2021). This ability to form resilient biofilms directly contributes to the widespread and persistent contamination observed across diverse settings. Table 2 summarises major reported cases of *B. cereus* contamination across food and clinical settings, highlighting the persistence of this organism in dairy and starchy foods.

Antibiotic stress, particularly from aminoglycosides, can induce certain *B. cereus* strains to transition into small colony variant (SCV) phenotypes. It is characterized by slower growth, enhanced toxin production, and elevated antimicrobial resistance (Frenzel et al., 2015). A growing body of evidence indicates that biofilm formation in *B. cereus* is intricately linked with its sporulation capacity, with both processes governed by shared genetic pathways. Central to this regulation are genes such as *SpoVG*, *Spo0A*, and *comER*, which coordinate the initiation of biofilm formation and spore development. The SinI–SinR circuit, along with matrix-associated genes *tasA* and *sipW*, further contributes to biofilm structural stability and maturation (Caro-Astorga et al., 2020; Lin et al., 2022). Notably, several natural agents including citral and terpinen-4-ol have demonstrated dual-action capabilities by disrupting quorum sensing, inhibiting sporulation-associated gene expression, and reducing biomass accumulation, thereby offering promising strategies for biofilm-targeted interventions in food safety systems (Kalia et al., 2023).

Beyond developmental regulators, quorum sensing (QS) plays a pivotal role in synchronising biofilm formation, virulence expression, and stress adaptation in *B. cereus*. Multiple QS systems, including the PlcR–PapR and LuxS/AI-2 pathways, interact with global transcriptional regulators to coordinate community-level behaviours essential for biofilm maturation. Xu et al. (2025) demonstrated that disruption of these QS circuits using sub-inhibitory concentrations of hordenine significantly suppressed QS gene expression and reduced toxin production, highlighting the importance of QS signalling in maintaining structured biofilms. Importantly, QS pathways intersect with Spo0A-centred regulation. Spo0A functions as a master developmental switch linking QS signals with cellular decisions governing biofilm formation and sporulation. Xu et al. (2017) showed that Spo0A functions as a master developmental regulator essential for structured biofilm formation in *B. cereus* AR156, acting through the *spo0A, sinI and sinR* regulatory circuit to drive matrix production, extracellular fibre assembly, and cellular differentiation required for mature biofilm architecture. Together, these findings suggest that QS signalling and Spo0A-mediated developmental pathways are tightly interconnected, with QS cues modulating Spo0A-dependent cell-fate decisions that balance biofilm maturation and sporulation.

Environmental stressors such as nutrient limitation, oxidative stress, and antimicrobial exposure activate upstream regulators including AbrB, SpoVG, and ComER, modulating the phosphorylation and activation of Spo0A (Huang et al., 2021; Marathe et al., 2023). Once activated, phosphorylated Spo0A (Spo0A ~ P) simultaneously promotes biofilm matrix production and initiates sporulation, a dual role reinforced through the SinI–SinR system and matrix proteins TasA and SipW (Bianco et al., 2020; Lin et al., 2022). Mature biofilms therefore act not only as protective niches for vegetative cells but also as

Table 1
Notable foodborne illness outbreaks (2017–2024): pathogens, contamination sources, and public health impacts.

Year	Country/Region	Pathogen	Source of contamination	Impact	Reference
2017	Korea	*Vibrio parahaemolyticus*	Squid	237 symptoms, 53 hospitalisations	Jung (2018)
2017–2019	Canada	*Salmonella* spp.	Frozen raw breaded chicken	68 cases	Kerr et al. (2024)
2021	China	*B. cereus*	Poor storage of rice noodles	198 cases	Li et al. (2023)
2022	Australia	*Campylobacter*	Duck liver	20 cases	McAllister et al. (2023)
2023	Indonesia	*E. coli*	Fried chicken	68 cases	Iskandar et al. (2025a, 2025b)
2024	USA	*L. monocytogenes*	Deli-sliced meat	61 cases	Sharma et al. (2025)

Table 2
Reported *B. cereus* contamination events in food.

Year	Study/Case	Country	Source of Contamination	Impact	Reference
2012	Rice outbreak in primary school	Malaysia	Nasi kuning	33 from 188 had upper gastrointestinal symptom	Jeffree and Mihat (2016)
2021	Foodborne outbreak in middle school	China	Rice noodle	198 cases	Li et al. (2023)
2021	Outbreak in boarding school	Malaysia	Beef rendang	152 cases	Bujang et al. (2023)
2023	Outbreak in school	Uganda	Canteen food	267 cases	Namara et al. (2025)
2024	Outbreak in elementary school	Indonesia	Snacks	12 cases	Handika et al. (2024)

microenvironments conducive to spore formation, particularly under harsh food-processing conditions (Marmion et al., 2022). Fig. 1 summarises the synergistic relationship between biofilm formation and sporulation in *B. cereus* under environmental stress.

Crucially, disruption of the *Spo0A*-centred pathways results in compromised biofilm integrity and decreased spore yield, thereby reducing the persistence of *B. cereus* in food-related environments (Lin et al., 2022). This interconnected regulatory axis presents a strategic target for antimicrobial interventions. Natural compounds such as citral have emerged as potential dual-action agents, capable of impairing both biofilm formation and sporulation gene expression (Almatroudi, 2024). A deeper understanding of this regulatory circuitry is essential for developing innovative and integrated approaches to mitigate foodborne contamination and ensure safer food processing systems.

1.2.2. *Spore formation and thermal resistance*

Spore formation in *B. cereus* serves as a key survival strategy, allowing the bacterium to endure extreme environmental conditions. Spores are highly resistant to heat, ultraviolet radiation, desiccation, commercial sanitizers, and food preservatives such as nitrites, sulfites, and organic acids. This resistance is largely due to their specialized structure, including a thick spore coat, cortex, and inner membrane, which collectively protect the spore's core components (Zegeye et al., 2021).

Recent studies show that the environment in which spores are formed can influence how resistant they become. Kim et al. (2024) found that when *B. cereus* sporulates in media with higher NaCl concentrations, the resulting spores have lower heat resistance. They also observed that high-salt conditions reduce endospore hydrophobicity, membrane fluidity, and spore density. This suggests that salinity disrupts key structural and physicochemical features essential for maintaining spore robustness.

Furthermore, studies from Lamba et al. (2022) shown that spores embedded within biofilms possess greater resistance to heat treatments compared to their planktonic cells. During biofilm maturation, environmental stresses such as nutrient limitation and oxygen depletion can activate sporulation pathways, leading a subset of cells to differentiate into spores within the biofilm matrix. This dual survival strategy enables the bacterial population to persist under hostile conditions. The biofilm matrix further acts as a physical barrier, shielding the spores from heat and commercial sanitizers, thereby preserving their viability. This finding underscores the importance of biofilm control strategies in mitigating spore-mediated contamination in food-processing environments (Liu et al., 2023).

Given the variability in strain behaviour and the global distribution of *B. cereus*, regulatory guidelines vary across regions. Table 3 presents selected international standards and regulatory thresholds for acceptable *B. cereus* levels in food.

Effective *B. cereus* control in food systems must integrate environmental monitoring, advanced cleaning technologies, and molecular detection methods for high-risk strains. Harmonisation of international standards remains a long-term goal, yet practical progress has been made through the adoption of molecular surveillance tools in countries like China, Poland, Australia, New Zealand, America and within the EU.

1.3. *Limitations of synthetic preservatives and demand for natural alternatives*

Traditional food preservation techniques such as chemical preservatives, thermal processing, and refrigeration have long been fundamental to maintaining food safety, extending shelf life, and preserving food quality. Chemical preservatives like nitrites, sulfites, and sorbates inhibit microbial growth and delay spoilage. Similarly, pasteurization and sterilization eliminate pathogens and enzymes, while refrigeration reduces microbial activity to prolong freshness (Ariyamuthu et al., 2022).

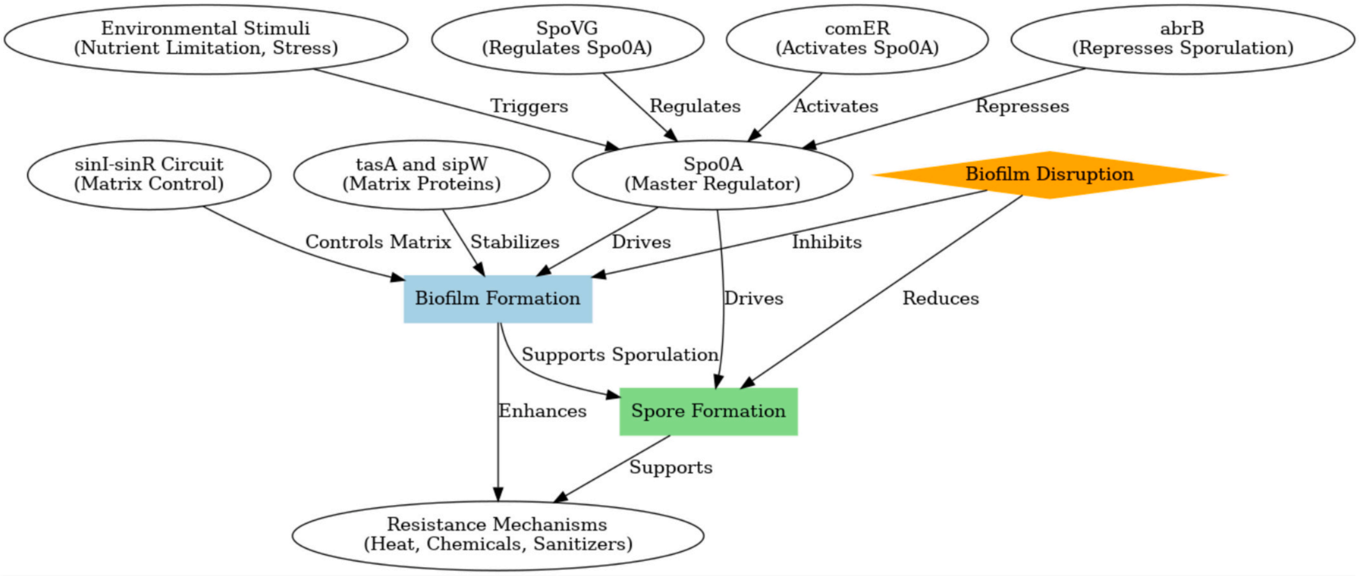

Fig. 1. Regulatory network linking biofilm formation and sporulation in *B. cereus*.

Table 3
International regulatory limits on *B. cereus* contamination.

Region/ country	Threshold (CFU/g)	Details	Reference
European Union	$\leq 10^3$	Limit applies to ready-to-eat foods at end of shelf life to prevent toxin production.	Berthold-Pluta et al. (2019)
United States	$\geq 10^5$	The United States does not specify a regulatory limit for *B. cereus*; values shown reflect levels commonly associated with illness in outbreak investigations.	McDowell et al. (2023)
China	10^3–10^5	No official threshold: virulence genes are monitored using molecular tools.	Gao et al. (2018)
Australia/ New Zealand	$\leq 10^3$	Regulatory agencies reject foods above this level.	FSANZ (2013)
Poland	$\leq 10^3$	Emphasis on routine monitoring of processed food for spore-forming bacteria.	Berthold-Pluta et al. (2019)
Singapore	$< 10^2$	Microbiological standards for ready-to-eat-food must be less than this level.	Rusnan et al. (2020)

However, these methods are increasingly questioned due to health, regulatory, and sustainability concerns. Nitrites have been linked to carcinogenic nitrosamine formation, while sulfites can trigger allergic reactions in sensitive individuals (Shakil et al., 2022). Thermal treatments may degrade heat-sensitive nutrients (e.g., vitamin C) and alter flavour or texture, potentially reducing consumer appeal. Refrigeration, although effective, demands high energy use, which poses challenges in resource-limited or rural areas (Lisboa et al., 2024). Consumer preferences have shifted notably in recent years, favouring "clean label" products that exclude synthetic additives. This shift is driven by growing awareness of health impacts, environmental sustainability, and interest in minimally processed foods (Asioli et al., 2017). Natural preservation methods, particularly those involving plant-derived compounds, antimicrobial peptides, and non-thermal technologies like supercritical fluid (SFE) extraction are now widely explored as alternatives (Li et al., 2024).

Studies across various sectors support this trend. In the meat industry, polyphenol-rich plant extracts are being adopted to replace synthetic preservatives (Beya et al., 2021). In seafood, natural agents such as essential oils and chitosan extend shelf life while satisfying consumer demands for chemical-free preservation (Olatunde and Benjakul, 2018). While natural preservatives are generally well accepted by consumers, some of it can cause pronounced sensory changes, including bitterness and harsh flavour notes, particularly at higher concentrations. This sensory limitation often constrains their application in commercial food products, as producers must balance antimicrobial efficacy with flavour acceptability. For example, propolis and spice extracts introduced slight variations in color or flavour in meat products but did not significantly affect consumer acceptance when used at optimal concentrations (Pobiega et al., 2019; Procopio et al., 2022). Natural preservatives sourced from plants (e.g., citral from lemongrass), animals (e.g., lysozyme, chitosan), and microbes (e.g., nisin) are often biodegradable, broadly antimicrobial, and less likely to promote resistance. Many also offer added antioxidant and sensory benefits, enhancing both food safety and quality (Barberis et al., 2018; Quinto et al., 2019; El-Saber Batiha et al., 2021). Fig. 2 provides an overview of the classification of food preservation highlighting synthetic and the diversity of natural preservatives.

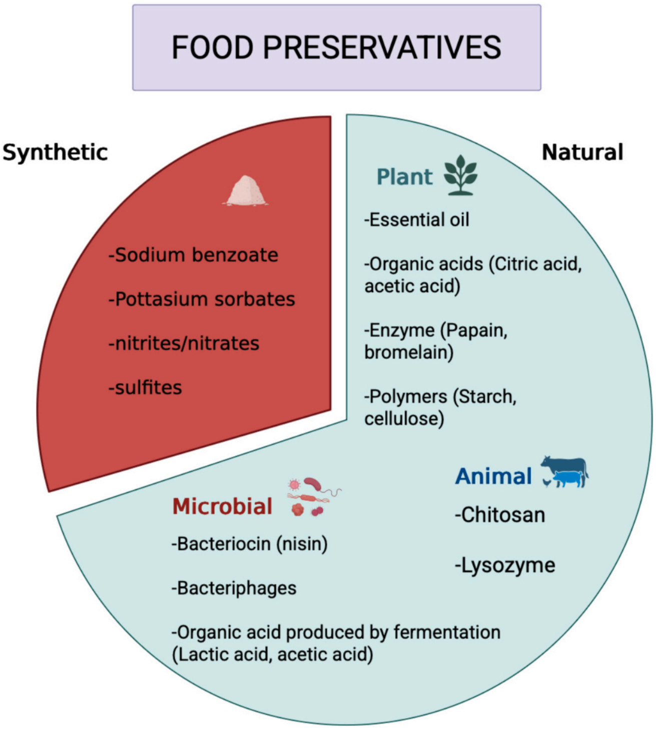

Fig. 2. Classification of food preservatives.

The growing limitations of synthetic preservatives combined with shifting consumer demands for safe, natural, and sustainable food systems have accelerated research into natural alternatives Given these limitations and consumer trends, there is growing interest in plant-derived preservatives such as lemongrass, which offers promising antimicrobial potential alongside cultural familiarity and wide availability in regions like Malaysia.

1.4. *Recent developments of plant-derived antimicrobials and essential oils*

Recent research has substantially advanced the understanding of plant-derived antimicrobials, particularly essential oils (EOs), as effective natural alternatives for food safety applications. Recent evidence indicates that essential oils exert antimicrobial activity through multiple, concurrent mechanisms, including disruption of microbial cell membranes, interference with energy metabolism, induction of oxidative stress, and modulation of key regulatory pathways associated with virulence and biofilm formation (Iskandar et al., 2025a, 2025b). For example, Gao et al. (2024) demonstrated that *Angelica sinensis* essential oil significantly increased membrane permeability in *Penicillium roqueforti*, inhibited sterol biosynthesis pathways, and activated stress-response and apoptotic signalling, resulting in effective fungal inactivation and extension of bread shelf life.

Building on these foundational mechanistic insights, recent studies have further expanded understanding by integrating omics-based approaches with application-oriented delivery strategies. Feng et al. (2025) reported that synergistic combinations of essential oil components, such as citral and eugenol, triggered mitochondrial dysfunction, reactive oxygen species accumulation, DNA degradation, and metacaspase-dependent apoptosis in *Aspergillus niger*, while microencapsulation of these compounds enabled controlled release and significantly prolonged the shelf life of baked products. Similarly, Yang et al. (2025) highlighted that essential oils can simultaneously target membrane integrity, metabolic pathways, and stress-response networks in foodborne pathogens, supporting their effectiveness at relatively low concentrations when appropriately formulated.

In parallel, Jacob et al. (2025) further highlighted that the successful translation of essential oils into food preservation systems is increasingly governed by material–formulation design rather than antimicrobial potency alone**.** Their analysis demonstrated that nano-enabled delivery platforms such as nanoemulsions, polymeric nanofibres, and biopolymer-based encapsulation matrices not only improve aqueous dispersibility and physicochemical stability of essential oils, but also enable controlled and sustained release at the food–microbe interface, thereby enhancing antimicrobial efficacy at reduced dosages.

Collectively, these recent advances signify a clear paradigm shift from the use of essential oils as simple antimicrobial additives toward their recognition as multi-target, formulation-dependent bioactive systems, capable of achieving effective microbial control while mitigating sensory limitations and aligning with clean-label and sustainability requirements. Within this broader context of recent advances in plant-derived antimicrobials, lemongrass essential oil has emerged as a particularly promising candidate due to its high citral content and well-documented antimicrobial efficacy.

2. Lemongrass *(C. citratus)*: botanical and ethnopharmacological background

2.1. Botanical description, distribution, and cultivation in Malaysia

C. citratus, commonly known as lemongrass, is a perennial, aromatic grass belonging to the *Poaceae* family, which includes over 600 genera and 10,000 species (Aćimović et al., 2019). It grows in dense clumps, reaching up to 2 m in height, with long, narrow, linear leaves and a rhizomatous root system. The plant releases a strong lemon-like fragrance due to the presence of citral, a key bioactive compound known for its potent antimicrobial properties (Kamaruddin et al., 2021; Shelar et al., 2023). Owing to these attributes, lemongrass is widely valued for its use in culinary applications, traditional herbal medicine, aromatherapy, and essential oil extraction (Ashaq et al., 2024).

Although *lemongrass* is commonly believed to have originated in India and Sri Lanka, several sources suggest Malaysia as a possible native region due to its long-standing use and cultivation (Majewska et al., 2019). Today, *lemongrass* is extensively cultivated in tropical and subtropical regions, including Southeast Asia, Central and South America, Africa, and the Indian Ocean islands (Wifek et al., 2016). In Malaysia, it is grown for both domestic and commercial reasons, particularly in the states of Johor, Pahang, and Negeri Sembilan. The plant thrives in warm, humid climates with well-drained soils and full sunlight exposure, and it is typically propagated through vegetative division of clumps rather than seeds (Shahrul, 2022).

Given Malaysia's favourable climate and established agricultural practices, the country has seen progressive expansion in lemongrass cultivation over recent years, both in terms of land area and production output. According to the Department of Agriculture Malaysia's Agrofood Statistics Reports, lemongrass cultivation has shown steady growth in both planted area and production volume from 2017 to 2022. In 2022 alone, the total planted area reached 1715.8 ha, with a production output of 16,404.7 metric tons and an average yield of 10.7 metric tons per hectare. Notably, the production value in 2022 was estimated at 37.7 million RM (Ringgit Malaysia) equivalent to 8.9 million USD, reflecting a strong market demand for lemongrass as both a culinary and commercial crop. Table 4 presents a year-by-year breakdown of cultivation area, yield, production volume, and economic value over the six-year period (KPKM, 2023).

Lemongrass is also widely recognized under various common names reflecting its cultural reach: "lemongrass" or "West Indian lemongrass" in English, "citronnelle" in French, "hierba limón" in Spanish, "capim-santo" in Portuguese, "xiang mao" in Chinese, and "serai" in Malay. This diversity in nomenclature signifies the plant's broad global relevance in culinary and medicinal contexts (Rojas-Sandoval, 2016).

2.2. Traditional culinary and medicinal uses of lemongrass

Lemongrass is a versatile herb widely used across Southeast Asian cuisines, particularly in Malaysia, Indonesia, and Thailand. Its distinctive citrus aroma and flavour enhance a variety of culinary preparations, including soups, curries, stir-fries, and marinades. Notably, it features prominently in signature regional dishes such as *tom yum* and *tom kha kai*, where its bruised stalks are simmered to infuse the broth with aromatic oils (Gaba et al., 2020). Beyond its culinary appeal, lemongrass is also traditionally consumed as a herbal infusion and often prepared with ginger or mint to aid digestion, relieve bloating, and alleviate symptoms of colds and flu (Kassahun et al., 2020).

The medicinal significance of *lemongrass* is equally notable. In traditional medicine systems across Southeast Asia, lemongrass has long been used as a folk remedy for multiple therapeutic purposes, including coughs, fever, gingivitis, headaches, leprosy, malaria, respiratory infections, and vascular disorders (Gaba et al., 2020). In Malaysia and Indonesia, a decoction made by boiling lemongrass is traditionally consumed to enhance blood circulation, particularly in the pelvic and uterine regions (Kassahun et al., 2020). In addition, the herb is widely used as a natural insect repellent and insecticide, particularly in rural communities where chemical alternatives are less accessible (Kassahun et al., 2020).

Recent ethnobotanical studies from India highlight lemongrass's broad-spectrum medicinal potential. Traditionally, it is used to treat gastrointestinal issues such as stomach aches, constipation, and diarrhoea. Its antipyretic and profuse sweating effects make it effective for reducing fevers by promoting perspiration (Mukherjee et al., 2024). The essential oil derived from *lemongrass* has demonstrated significant antimicrobial, antifungal, and anti-inflammatory activity, underscoring its importance across various traditional healing systems (Boukhatem et al., 2014).

3. Bioactive properties of lemongrass essential oil

3.1. Antimicrobial activity against foodborne pathogens

Lemongrass essential oil (LEO) demonstrates potent antimicrobial activity, primarily due to its high citral content a mixture of the isomer's geranial and neral. Citral disrupts bacterial cytoplasmic membranes, increases membrane permeability, and causes intracellular leakage, leading to rapid cell death (Valková et al., 2022). This activity is particularly effective against Gram-positive foodborne pathogens such as *B. cereus* and *Staphylococcus aureus*. Although clove oil exhibited higher overall potency in comparative studies, citral showed moderate inhibitory effects on *E. coli* compared to *B. cereus* (Gutierrez-Pacheco et al., 2023). Its preferential efficacy against Gram-positive bacteria is

Table 4

Lemongrass cultivation area, production volume, and economic value in Malaysia (2017–2022).

Year	Planted Area (ha)	Production Area (ha)	Average Yield (mt/ha)	Production (mt)	Production Value (RM '000)	Production Value (USD '000)
2017	1818.0	1493.6	9.2	13,674.2	40,019.90	9471.26
2018	1594.4	1297.3	9.8	12,767.7	29,365.75	6950.25
2019	1418.5	1274.0	9.7	12,332.0	30,213.34	7151.30
2020	1695.3	1673.1	9.5	15,818.0	36,381.39	8610.65
2021	1981.3	1840.5	10.1	18,585.0	40,886.99	9677.47
2022	1715.8	1532.8	10.7	16,404.7	37,687.95	8920.35

attributed to their simpler cell wall structure, in contrast to the outer lipopolysaccharide layer of Gram-negative bacteria, which impedes the penetration of hydrophobic essential oil components (Budiati et al., 2018; Subramaniam et al., 2020).

In addition to inhibiting planktonic cells, citral has also been shown to suppress spore germination and vegetative outgrowth of *B. cereus*, further supporting its role as a natural preservative in high-risk foods (Wang et al., 2024b). In dairy applications, LEO has shown moderate antimicrobial activity against multidrug-resistant strains such as *E. coli*, *Staphylococcus* spp., and *Enterobacter*, although its effectiveness against *Klebsiella* remains limited (Yasir et al., 2022).

Mechanistically, citral exerts its effects through multiple cellular targets. It disrupts the cytoplasmic membrane (1), causes leakage of intracellular contents (2), interferes with DNA replication and transcription (3), and induces protein denaturation (4). Enzymatic inhibition (5) and impaired protein synthesis (6). In Gram-negative bacteria, citral can penetrate the lipopolysaccharide layer or form pores in the outer membrane (7), allowing access to internal cellular components (Shi et al., 2016; Gutierrez-Pacheco et al., 2023). These proposed cellular mechanisms are visually summarised in Fig. 3. When delivered via nanoemulsion systems, these antimicrobial effects are further enhanced through improved dispersion, increased droplet–cell contact frequency, and sustained release at the bacterial surface, as illustrated in Fig. 4.

These mechanistic insights are consistent with the findings of Alhantoobi et al. (2025), who reported that hydrophobic constituents of essential oils interact directly with bacterial cell envelopes, resulting in membrane destabilization, enhanced permeability, and leakage of intracellular components. Such primary disruptions subsequently impair essential cellular functions, including energy metabolism, nucleic acid synthesis, protein activity, and enzymatic pathways. Moreover, essential oils have been shown to interfere with bacterial regulatory systems, such as quorum sensing and stress-response pathways, thereby reducing biofilm formation and persistence (Guillín et al., 2021). This multimodal mode of action underpins the broad-spectrum antimicrobial efficacy of essential oils and distinguishes them from conventional antibiotics.

3.2. *Anti-biofilm activity of lemongrass oil*

Biofilm formation is a key survival strategy for many foodborne pathogens, including *B. cereus*, enabling persistence on food contact surfaces and resistance to sanitization. LEO, rich in citral, has shown promising anti-biofilm properties, including disruption of biofilm matrix

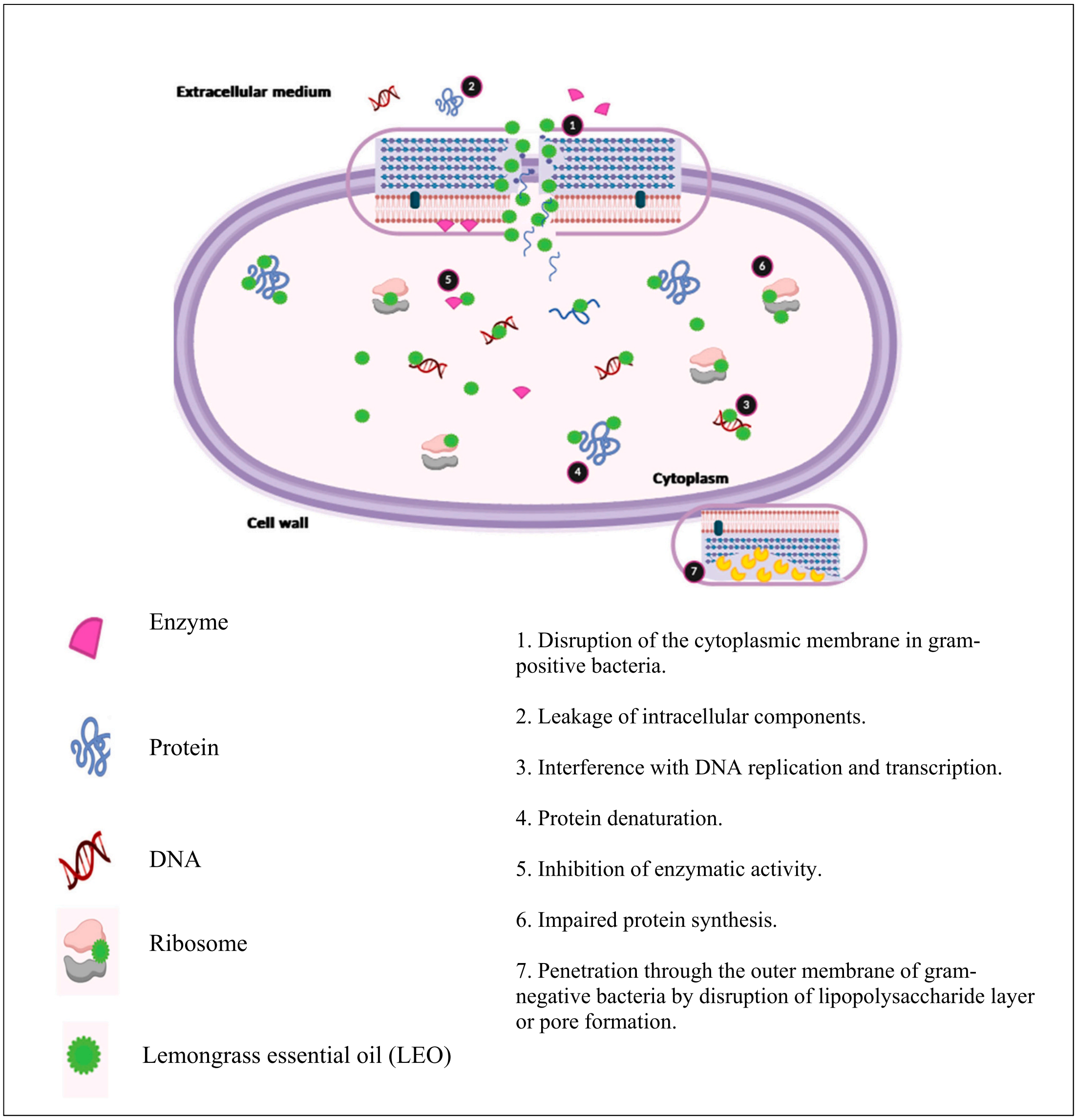

Fig. 3. Proposed mechanism of antimicrobial action of lemongrass essential oil (LEO) against gram-positive and gram-negative bacteria.

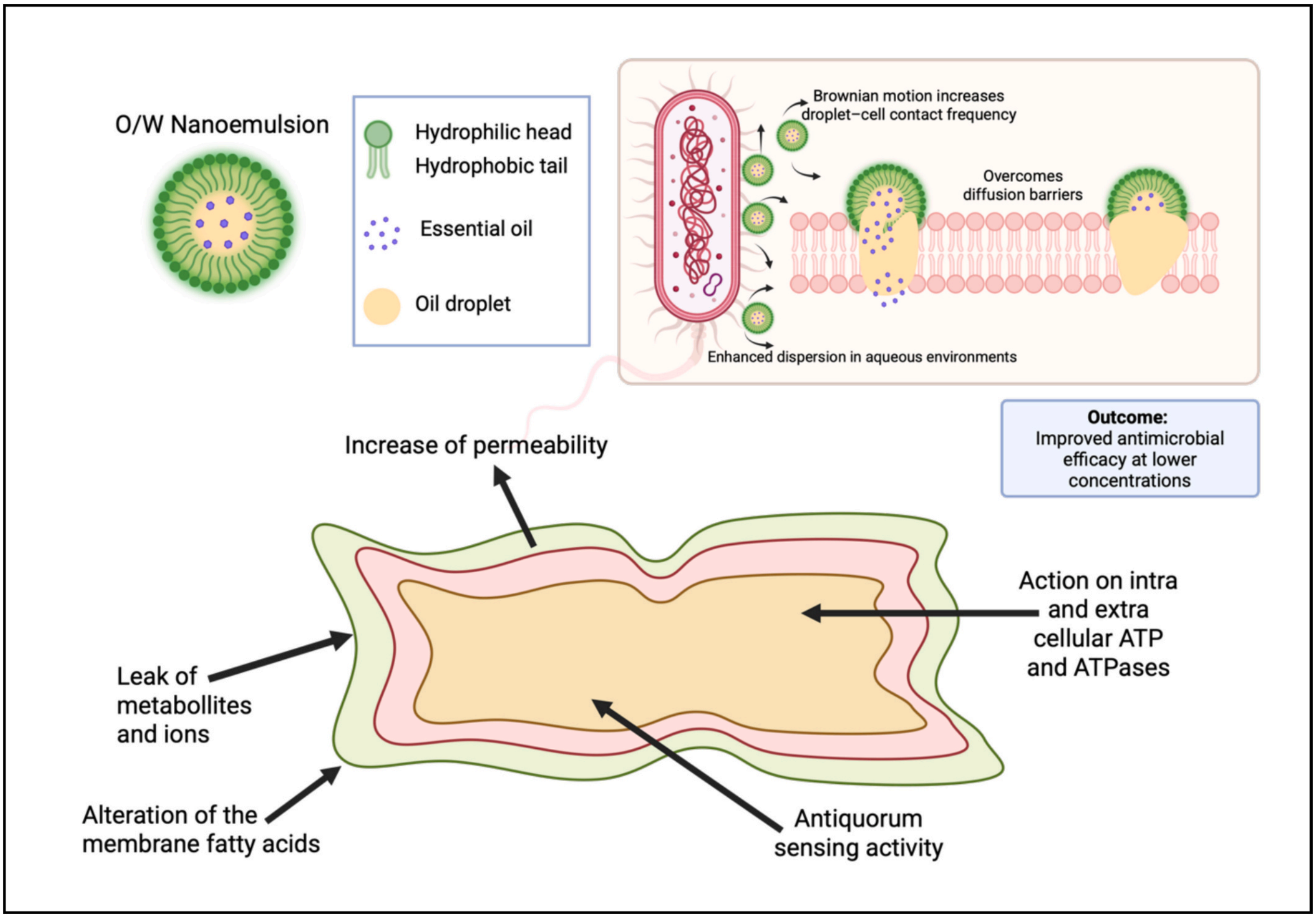

Fig. 4. Schematic illustration of nanoemulsion-mediated delivery of lemongrass essential oil against foodborne pathogens. Nanoemulsions enhance aqueous dispersion, increase droplet–cell contact through Brownian motion, overcome diffusion barriers at the bacterial envelope, and enable sustained release of citral at the cell surface, collectively improving antimicrobial efficacy at lower concentrations.

structure and inhibition of quorum sensing signals essential for microbial communication and virulence (Mukarram et al., 2021). These stage-specific anti-biofilm actions are schematically illustrated in Fig. 5.

Consistent with these mechanisms, multiple studies have demonstrated that LEO significantly inhibits early biofilm development and reduces biofilm biomass. Gao et al. (2020) reported that citral concentrations as low as 0.0781% effectively inhibited the planktonic growth of *S. aureus*, *Candida albicans*, and *Candida tropicalis*, while 0.3125% LEO disrupted dual-species biofilms by targeting biofilm structural integrity and signalling pathways. Supporting these findings, Karimou et al. (2024) further demonstrated the anti-biofilm potential of *lemongrass* extracts. In their study, aqueous extracts of *lemongrass* inhibited biofilm formation of *E. coli* by up to 50.1% and *Staphylococcus* spp. by up to 48.56%. Notably, the highest inhibition against *E. coli* (50.1%) and *Staphylococcus* spp. (48.56%) was observed with the aqueous *lemongrass* extract, whereas ethanolic extracts were generally less effective. Adukwu et al. (2012) further highlighted citral's potent activity, observing complete inhibition of *S. aureus* biofilm formation at concentrations as low as 0.060–0.125% (*v*/v). However, LEO was less effective in eradicating mature, established biofilms, indicating its role may be more preventive than curative. Interestingly, while citral alone showed strong inhibition zones (>8.6 cm), whole LEO sometimes performed better due to synergistic effects among minor components.

Combination treatments have also shown promise. Oliveira et al. (2010) reported that a mixture of *Cymbopogon nardus* and *lemongrass* oils eliminated adhered cells in 240 h old biofilms after only 60 min of contact, demonstrating enhanced efficacy in complex matrices. Nonetheless, results can vary across species and concentrations. Leonard et al. (2010) showed that while certain essential oils reduced *L. monocytogenes* biofilm biomass, lemongrass oil was also associated with biofilm enhancement rather than inhibition. These contrasting findings emphasize the need for careful optimisation of essential oil selection and application in food systems, as inappropriate use may unintentionally promote biofilm development. Despite its generally higher efficacy against bacterial biofilms, fungal biofilms tend to be more resilient to LEO. Andrade-Ochoa et al. (2021) reported that fungi were more resistant compared to bacterial species, while Adebayo and Osuolale (2024) confirmed the antifungal efficacy of LEO against *Candida* species, further supporting its broad-spectrum application in food spoilage prevention.

Lemongrass oil's ability to interfere with biofilm development and quorum sensing offers a promising natural strategy for controlling *B. cereus* contamination in food systems. Its preventative role is particularly valuable in environments prone to biofilm accumulation, such as dairy processing lines, packaging surfaces, and ready-to-eat food facilities.

3.3. *Anti-spore effects and food preservation potential*

Bacterial spores, particularly those of *B. cereus*, present significant challenges in food safety due to their resilience against conventional inactivation methods. Traditional approaches such as high-pressure processing, irradiation, and recent treatment cold plasma low pressure, are often effective but can compromise food quality and nutritional value (Kim et al., 2019; Mok et al., 2022; Valdez-Narvaez et al., 2024).

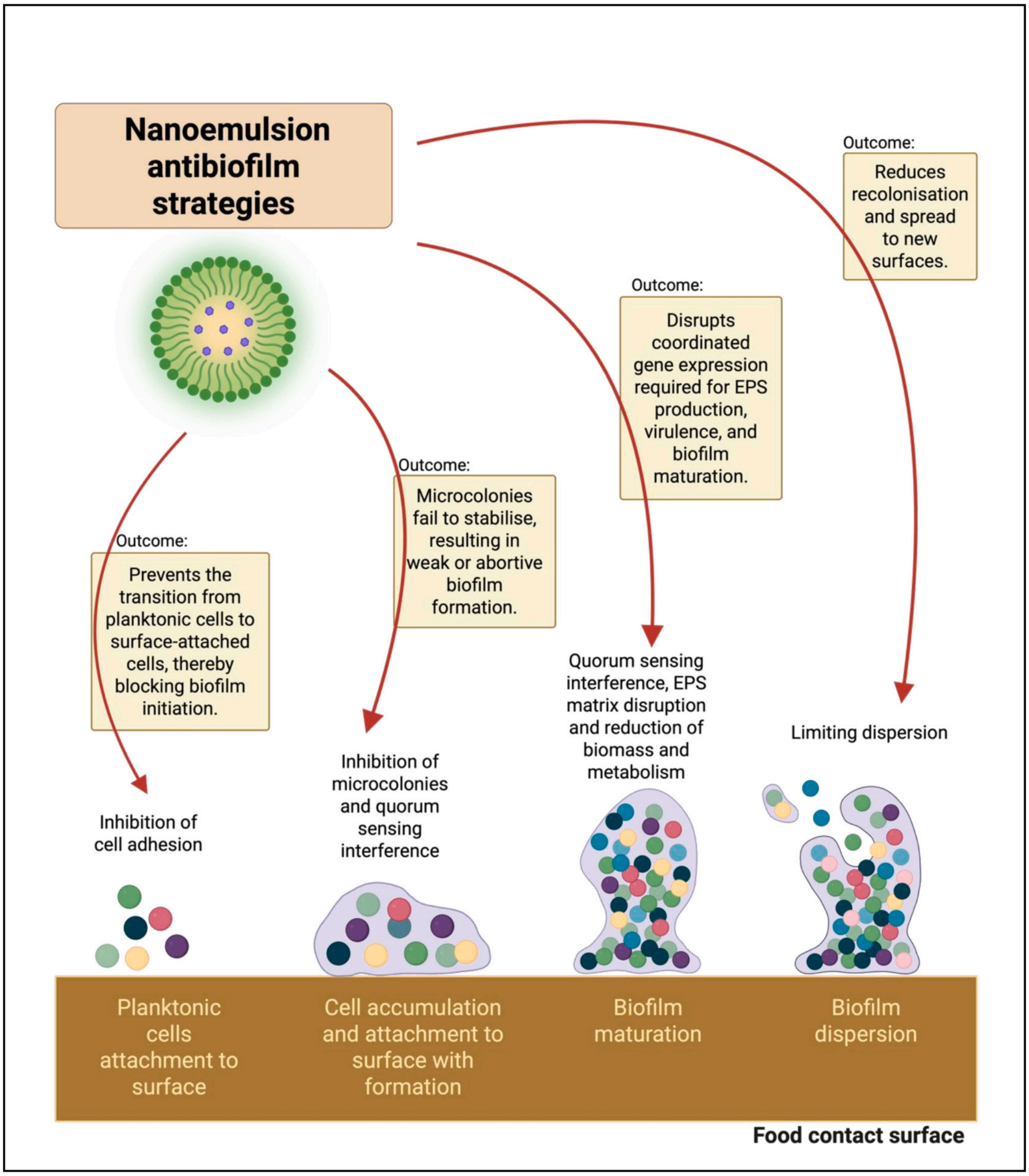

Fig. 5. Schematic representation of nanoemulsion-mediated anti-biofilm strategies of lemongrass essential oil. Nanoemulsions interfere with multiple stages of biofilm development, including inhibition of planktonic cell attachment, prevention of microcolony stabilisation, disruption of quorum sensing and EPS production during biofilm maturation, and reduction of biofilm dispersion and recolonisation on food contact surfaces.

Consequently, natural antimicrobials like LEO are gaining attention as safer, more sustainable alternatives.

In laboratory settings, LEO has demonstrated promising antibacterial spore activity. Schweitzer et al. (2022) reported that citral, the major component of LEO, significantly reduced the germination and outgrowth of *B. thuringiensis* spores by compromising membrane integrity and inhibiting early germination events.

4. Extraction and characterization of lemongrass essential oil

4.1. Overview of conventional and modern extraction methods

The extraction of LEO has undergone significant advancements to improve yield, preserve bioactive compounds, and enhance sustainability. Among the traditional methods, steam distillation remains the most widely used due to its simplicity, low cost, and environmental friendliness. It is particularly favoured for large-scale production as it effectively extracts essential oils without altering product quality. However, steam distillation can be time-consuming and may cause degradation of heat-sensitive components (Machado et al., 2022). Soxhlet extraction, another conventional approach, uses solvents such as ethanol to extract oil from plant materials. This method generally achieves higher yields than steam distillation but is limited by its long extraction time and high solvent consumption, which raises environmental and safety concerns (Okonkwo and Ohaeri, 2019).

To address these limitations, modern extraction techniques have been developed. Microwave-assisted hydrodistillation (MAHD) utilizes microwave energy to heat plant material rapidly and evenly, improving extraction efficiency and preserving volatile constituents. It has been shown to significantly reduce processing time while yielding essential oils of similar quality than conventional methods (Ghazanfari et al., 2020). Another technique, ultrasound-assisted extraction (UAE), employs ultrasonic waves to disrupt plant cell walls, facilitating the release of intracellular oils. UAE has proven particularly effective in increasing extraction yields and reducing solvent usage (Shen et al., 2023).

In recent years, supercritical fluid extraction (SFE) using carbon dioxide (CO_2) has gained prominence as a green technology in essential oil extraction. Operating under moderate temperatures and high pressures, SFE preserves heat-sensitive compounds and produces citral-rich extracts with superior purity and yield. (Ashaq et al., 2024) demonstrated that optimized SFE conditions lead to efficient extraction of LEO with minimal thermal degradation, making it ideal for food and pharmaceutical applications.

The choice of extraction method affects the chemical profile and bioactivity of LEO. Parameters such as extraction time, temperature, solvent type, and plant material characteristics must be carefully optimized to meet industrial and therapeutic objectives. Recent studies emphasize that the application of modern, sustainable extraction techniques not only improves efficiency and safety but also enhances the functional properties of essential oil (Hedayati et al., 2025). To synthesise the key factors influencing essential oil extraction efficiency across different techniques, Table 5 summarises the main process parameters, underlying mechanisms, and representative research findings reported in the literature.

4.2. *Influence of the extraction technique on citral content and bioactivity*

The efficiency of extracting citral from *lemongrass* is strongly influenced by the extraction technique used, which also affects the oil's overall bioactivity. Citral, comprising the isomers neral and geranial, is a key component contributing to LEO's antimicrobial and antioxidant properties. Among the advanced techniques, ultrasonic-assisted extraction has been shown to produce high citral content, with Yuniarto et al. (2022) reporting a yield of 83.15%, while microwave-assisted hydrodistillation yielded 68.32% of citral. Supercritical fluid extraction remains one of the most effective methods, achieving up to 84.95% citral content likely due to its low thermal degradation and efficient penetration of plant matrices (Carlson et al., 2001). In contrast, traditional hydrodistillation methods yield significantly lower citral concentrations. For instance, Jaleel et al. (2017) reported a citral content of only 43.1%, which may be attributed to thermal decomposition during prolonged heating. Vacuum distillation offers a moderate yield, with Viktorova et al. (2020) reporting a citral concentration of 63%, suggesting that reduced pressure can minimize degradation but may not maximize extraction efficiency. These findings collectively highlight that advanced, non-thermal extraction methods such as supercritical fluid and ultrasonic-assisted techniques not only preserve citral content more effectively but also enhance the potential bioactivity of the essential oil.

Overall, the literature indicates that essential oil extraction efficiency can be improved when process conditions promote effective mass transfer while minimising thermal and oxidative degradation. This is typically achieved under low-thermal or non-thermal conditions, such as supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted hydrodistillation, particularly when extraction time and temperature are optimized. Additional improvements are observed when appropriate pre-treatments are applied to disrupt plant cell structures and when pressure is carefully controlled to enhance solvent solvating power. These conditions are especially important for preserving thermolabile and volatile compounds such as citral.

4.3. *Stability and physicochemical profile of the extracted oil*

The stability of LEO, particularly its major active component citral, is influenced by environmental factors such as light, heat, oxygen, and moisture. These factors can lead to chemical degradation through oxidation, polymerization, isomerization, or hydrolysis, ultimately compromising the oil's therapeutic efficacy and sensory attributes. Studies have shown that prolonged storage reduces citral content; for instance, Akinkunmi et al. (2016) reported a drastic decline in citral content from 84.32% in fresh LEO to 2.39% after nine years of storage, along with reduced antimicrobial activity. The deterioration occurs because the chemical constituents of essential oils are prone to various reactions, including oxidation, isomerisation, polymerization, disproportionation, cyclisation, and dehydrogenation. Interestingly, new compounds were detected in the aged oil, indicating chemical changes over time. These findings highlight the need for proper storage such as protection from light and storage at low temperatures to preserve LEO's therapeutic quality (Rowshan et al., 2013; Turek and Stintzing, 2012).

Changes in citral content due to ageing and exposure to heat or light underscore the importance of controlled storage. Jenny et al. (2019) evaluated the physical stability of LEO-based microemulsions stored at 4 °C and 60 °C. The formulations maintained physical integrity over several months with no phase separation or creaming observed, though pH levels (4.1–5.1) suggested bacterial growth inhibition rather than fungal protection. Additionally, citral was shown to be more susceptible to degradation under acidic conditions. This is because low pH environments promote acid-catalyzed reactions such as isomerization and cyclization, leading to a breakdown of citral's chemical structure (Maswal and Dar, 2013).

Given citral's sensitivity to environmental degradation, ensuring the

Table 5
Factors affecting lemongrass essential oil extraction efficiency and citral retention.

Factor	Mechanism influencing extraction efficiency	Effect on yield and citral content	Representative research case
Extraction method	Determines heat exposure, mass transfer efficiency, and extent of cell wall disruption	Modern extraction techniques generally improve yield and better preserve citral compared to conventional methods	SFE (84.95% citral; Carlson et al., 2001); UAE (83.15%; Yuniarto et al., 2022); Hydrodistillation (43.1%; Jaleel et al., 2017)
Temperature	High temperatures enhance volatilisation but accelerate thermal degradation, oxidation, and isomerisation	Excessive heat reduces citral content and bioactivity	Reduced degradation reported for SFE and MAHD compared to hydrodistillation (Ashaq et al., 2024; Ghazanfari et al., 2020)
Extraction time	Prolonged extraction increases exposure to heat and oxygen after equilibrium is reached	Optimized extraction time improves efficiency; extended time reduces oil quality	Shorter extraction times in MAHD and UAE improve yield and composition (Ghazanfari et al., 2020; Shen et al., 2023)
Pressure (SFE)	Increased CO_2 density enhances solvating power and selectivity for non-polar compounds	Higher pressure improves recovery of citral-rich fractions	High citral recovery under optimized SFE pressure conditions (Carlson et al., 2001; Ashaq et al., 2024)
Plant material characteristics	Cell wall rigidity, oil gland density, moisture content affect solvent penetration and oil release	Proper selection and preparation enhance extraction efficiency	Influence of plant condition reported across extraction methods (Machado et al., 2022)
Pretreatment (e.g. ultrasound, microwave)	Disrupts plant cell walls and oil glands, enhancing mass transfer	Increases yield and reduces solvent and energy requirements	UAE improves yield and extraction efficiency (Shen et al., 2023)
Solvent type (conventional methods)	Solvent polarity determines extraction spectrum and efficiency	Organic solvents may increase yield but pose safety and environmental concerns	Soxhlet extraction achieves higher yield but with high solvent usage (Okonkwo and Ohaeri, 2019)

stability of essential oils is crucial for maintaining their bioactivity and shelf life. Nanoemulsions and encapsulation technologies have emerged as effective strategies to protect essential oils from heat, light, and oxidation. Their small droplet size and large surface area help entrap volatile compounds, reducing degradation. Ganosi et al. (2023) highlighted that such systems also enhance solubility and permeability, while extending shelf life. Mohd Daud et al. (2025) reported that both lemongrass and citral 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*. This shows that their effectiveness depends on formulation choices, particularly the type and concentration of surfactants and emulsifiers, which influence droplet size and system stability. Barradas and de Holanda e Silva (2020) noted, even under controlled storage, minor compounds may still degrade if the formulation is not fixed to the optimal standard. Therefore, optimal design of encapsulation systems is essential to maximize the stability of citral-rich oils.

5. Nanoformulation strategies

5.1. Nanoemulsion-based delivery enhancements for antimicrobial applications

The formulation and stability of LEO nanoemulsions are significantly influenced by the extraction method used to obtain the oil. Various studies have demonstrated that the choice of extraction technique not only affects citral content but also the physicochemical properties and bioactivity of the resulting nanoemulsion. Hebishy et al. (2022) employed supercritical fluid extraction (SFE), which preserved a high citral concentration and produced nanoemulsions with higher antimicrobial activity and stability. In contrast, Ayoub et al. (2023) and Saada et al. (2020) used hydrodistillation and produced stable nanoemulsions with average droplet sizes of 65–80 nm, although they exhibited lower citral retention. Similarly, Ali et al. (2023) formulated nanoemulsions from steam-distilled LEO, which were effective against *E. coli* and *S. aureus*, while Bezerra et al. (2023) applied microwave-assisted hydrodistillation (MAHD), resulting in better citral preservation and sustained antimicrobial action, particularly against L. *monocytogenes*.

Compared to conventional LEO formulations, nanoemulsions offer several advantages. Due to their small droplet size and increased surface area, nanoemulsions enhance solubility and bioavailability of hydrophobic compounds like citral. They also improve stability under environmental stressors (e.g., light, heat, oxygen), reduce volatility, and facilitate controlled release, which helps maintain antimicrobial and antioxidant efficacy over time. Furthermore, nanoemulsified LEO demonstrates greater dispersion in aqueous systems and stronger antimicrobial effects at lower concentrations compared to bulk oil (Ayoub et al., 2023; Hebishy et al., 2022).

Additional studies further validate these findings. Noorbakhsh et al. (2025) reported that LEO nanoemulsions produced using ultrasound-assisted emulsification showed excellent antimicrobial efficacy with droplet sizes below 100 nm and high kinetic stability under storage conditions. Gago et al. (2019) emphasized the relevance of nanoemulsion systems for food preservation, noting enhanced retention of sensory qualities and delayed microbial spoilage when lemongrass nanoemulsions were applied to fresh produce surfaces. Gonzalez et al. (2021) demonstrated that nanoemulsions incorporated into edible coatings significantly reduced pathogen load in fresh-cut fruit, confirming their utility in food packaging systems. Meanwhile, Salvia-Trujillo et al. (2012) found that nanoemulsions prepared with optimized surfactant-to-oil ratios promoted superior in vitro bioaccessibility and delivery of citral compared to macroemulsion systems, further supporting their functional advantages.

Overall, nanoemulsion technology enhances the functional potential of LEO as a natural antimicrobial agent. Its improved dispersion, storage stability, and pathogen-targeting capabilities support its application in food preservation and safety, as well as in broader health and environmental contexts.

5.2. Toxicity, biocompatibility, and safety in food applications

LEO, particularly its key compound citral, has demonstrated notable bioactivity alongside a generally favourable safety profile. Plata-Rueda et al. (2020) reported strong insecticidal effects of LEO, citral, and geranyl acetate against *Sitophilus granaries* (granary weevil), with low LD_{50} values ranging from 3.93 to 6.92 μg/insect and significant sublethal effects, including reduced respiration and mobility. In contrast, mammalian studies show low toxicity. Xavier et al. (2022) found no adverse outcomes following a 2000 mg/kg oral dose in rats, classifying both LEO and citral under Category 5 (lowest toxicity) of the Globally Harmonized System (GHS).

Complementing these findings, Weshahi et al. (2025) reported strong antioxidant activity in LEO extracted from an Omani cultivar. Although specific cytotoxicity data in mammalian models were not provided, the isolated oil demonstrated significant cytotoxic potential in a brine shrimp lethality assay, achieving 100% mortality at 1000 μg/mL.

To ensure consumer safety, LEO concentrations in food systems must be carefully optimized. Balancing efficacy and biocompatibility are crucial, especially considering its cytotoxicity at elevated doses. Regulatory guidelines provide an essential framework for establishing safe application levels in food industries.

6. Applications of lemongrass essential oil in food safety

6.1. As a natural preservative in food products (e.g., dairy, meat, grains)

Growing consumer demand for natural food preservatives has spotlighted LEO as a promising candidate, owing to its strong antimicrobial and antioxidant properties primarily driven by its citral content. Its application spans a variety of food matrices, including meat, tofu, dairy, beverages, and fresh produce. In tofu preservation, Hamad et al. (2019) demonstrated that a 20% water extract of lemongrass inhibited bacterial growth and extended shelf life by four days, while the essential oil component, although not antimicrobial in this context, helped maintain sensory qualities such as color, odor, and texture.

Advanced technologies such as encapsulation and nanoemulsion further enhance LEO's preservative effectiveness. Faheem et al. (2022) reported that nanoformulations of LEO combined with edible films (e.g., chitosan, alginate, or starch-based matrices) enhanced antimicrobial efficacy against pathogens like *E. coli*, *Salmonella*, and *L. monocytogenes*, while also improving the mechanical and barrier properties of packaging materials. In chicken meat preservation, Gan et al. (2024) encapsulated LEO in bilayer liposomes and demonstrated significant delays in spoilage indicators, such as total volatile nitrogen and bacterial load, extending shelf life from 7 to 12 days at 4 °C. The diverse applications of lemongrass essential oil nanoemulsions across food matrices and preservation strategies are schematically illustrated in Fig. 6.

Additionally, Iranloye et al. (2024) emphasized LEO's role as a broad-spectrum antimicrobial agent capable of targeting a variety of spoilage organisms across food types, supporting its integration in ready-to-eat and minimally processed foods. Applications have also extended to beverages and dairy, where lemongrass extract preserved freshness and inhibited microbial growth during storage.

Representative studies evaluating the application of lemongrass essential oil nanoemulsions in food preservation are summarised in Table 6, highlighting their antimicrobial efficacy, formulation strategies, and impact on shelf-life and sensory quality. Nevertheless, the performance of LEO can be affected by food composition and delivery methods, warranting further exploration into formulation technologies to optimize its use in industrial food preservation.

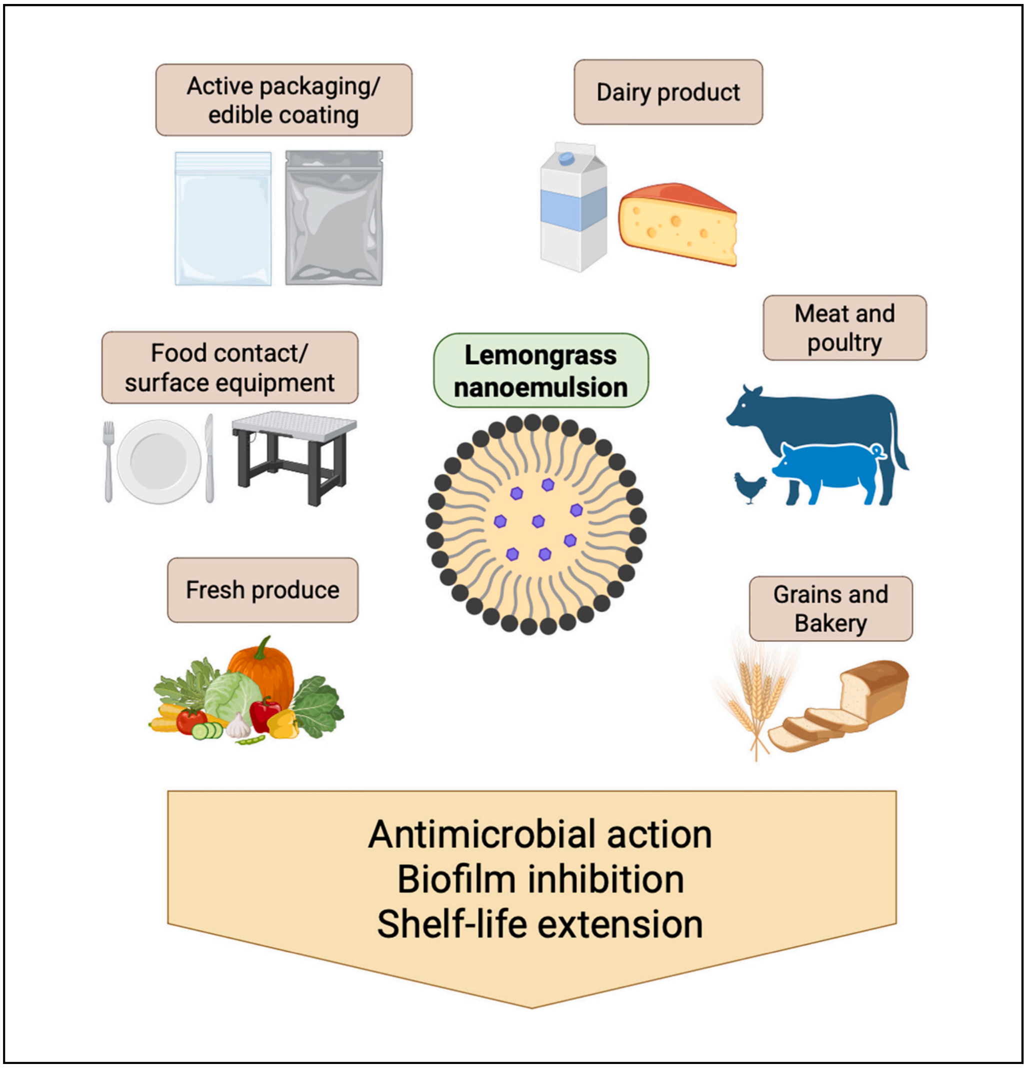

Fig. 6. Schematic overview of the application of lemongrass essential oil nanoemulsions in food preservation and safety systems. Lemongrass nanoemulsions can be incorporated into diverse food matrices, including dairy products, meat and poultry, grains and bakery items, and fresh produce, as well as into active packaging, edible coatings, and food-contact surfaces. These applications exploit the enhanced antimicrobial and anti-biofilm properties of nanoemulsified lemongrass essential oil to reduce microbial contamination and extend shelf life.

Table 6
Applications of lemongrass nanoemulsions in food preservation with antimicrobial impact across various food matrices.

Food product	Target microorganism	Emulsifier type	LEO concentration	Impact	Reference
Beef Burger	Total bacterial count (TBC)	Tween 80	1.0% and 1.5%	0.57 log reduction in TBC; improved sensory properties	Bakheet et al. (2024)
Chewy Candy	*Streptococcus mutans, Porphyromonas gingivalis*	Soy protein isolates and soy lecithin	Lemongrass to lemon ratio 1.85: 2.25.	85% inhibition of *S. mutans and* 77.20% inhibition of *P. gingivalis;* improved texture and color	Jayus et al. (2024)
Grape Berries	*S.* Typhimurium or *E. coli* O157:H7	Carnauba wax, Tween 80	2.0%	2 log reduction on *S. typhimurium* and 1 log reduction on *E. coli* O157:H7; maintained firmness and phenolic compound	Kim et al. (2014)
Pomegranate arils	*S.* Typhimurium or *E. coli* O157:H7	Flaxseed gum	800 ppm	4 log reduction in TPC; extended shelf life; maintained sensory attributes such as color, texture, and taste over 12 days of storage at 5 ± 1 °C.	Yousuf and Srivastava (2017)

6.2. *Use in surface sanitization and active food packaging*

Lemongrass extract and essential oil have demonstrated promising antimicrobial efficacy for surface sanitation in both healthcare and food industry contexts. Mathew et al. (2016) showed that lemongrass oil exhibited strong disinfectant properties against *E. coli* and *S. aureus* on floor surfaces comparable to commercial Lysol® disinfectant with a significant reduction in bacterial presence within just 10 min of application. Similarly, Sasi et al. (2020) assessed the sanitizing potential of lemongrass oil in meat processing environments and found it to significantly reduce total viable counts, coliforms, and yeast and mold loads on meat cutting boards ($p < 0.05$). Compared to other natural sanitizers like lemon juice and orange peel extract, lemongrass oil yielded the lowest microbial counts, suggesting its superior efficacy in reducing cross-contamination risks in hand hygiene applications.

Building on its versatility in antimicrobial applications, LEO has also gained substantial attention in the development of sustainable active packaging systems. Its potent antimicrobial and antioxidant properties make it well-suited for incorporation into biodegradable film matrices aimed at extending food shelf life and enhancing product safety. For example, Istiqomah et al. (2022) demonstrated that integrating LEO into a chitosan–*Dioscorea hispida* (Indian three-leaved yam) starch composite film significantly improved mechanical, thermal, and barrier properties while also exhibiting strong antibacterial activity against *E. coli*, *S. aureus*, *S*. Typhimurium, and *S. epidermidis*. Similarly, Ju et al. (2020) reported that citral-based microencapsulated sachet packaging enabled a controlled release of volatile compounds into the package headspace, effectively inhibiting mold and yeast growth and extending bread shelf life from 5 days to up to 15 days without compromising sensory quality, highlighting the effectiveness of citral as a volatile antimicrobial agent in active packaging systems.

Molecular docking analysis further revealed that the active components in LEO interact with bacterial FtsA enzymes through hydrogen bonding and hydrophobic interactions, suggesting a specific antibacterial mechanism. Similarly, Silva et al. (2025) applied a chitosan–LEO emulsion as a coating on paperboard and found enhanced thermal stability, hydrophobicity, and microbial resistance, particularly against insect infestation in stored grain products. The functional performance of these coated systems improved with increasing LEO concentration, although higher concentrations slightly affected cytotoxicity, indicating a need for optimal dosage balance.

In a separate study, Magri et al. (2025) developed LEO-loaded nanoemulsion films using gelatin as the carrier and reported extended shelf-life of perishable foods due to the controlled release of antimicrobial compounds. Jamroz et al. (2023) also confirmed this controlled-release profile in polylactic acid (PLA) based films, where the inclusion of LEO maintained antimicrobial efficacy without adversely impacting the sensory characteristics of food. Finally, Ruskova et al. (2023) highlighted the role of LEO in enhancing the multifunctionality of bio-based films, emphasizing its dual role as a natural preservative and an eco-friendly alternative to synthetic packaging additives. Collectively, these findings affirm that LEO not only improves the bioactivity of active packaging materials but also contributes to environmental sustainability in food preservation systems.

6.3. Sensory and organoleptic properties of lemongrass oil in food applications

Lemongrass and its essential oil are widely recognized for their distinctive citrus aroma and flavour, making them valuable natural additives across diverse food systems. Evidence from multiple studies demonstrates that lemongrass enhances flavour and aroma while maintaining desirable textural qualities, thereby improving overall sensory appeal and consumer acceptability.

In dairy applications, Krupa Joseph and Rao (2019) reported that incorporating lemongrass into paneer significantly improved its sensory profile. The addition of 4% (*w*/*v*) cut lemongrass leaves or 0.015% (*v*/*v*) lemongrass oil produced a pleasant lemony flavour and aroma with the highest sensory acceptability, whereas higher concentrations imparted grassy or harsh notes. Although lemongrass slightly altered the product's color, giving a faint greenish-yellow color at higher concentrations, it did not affect hardness, cohesiveness, or springiness, indicating that it can serve as a natural flavoring agent without compromising the structural integrity of dairy products.

In beverage applications, Lonkar et al. (2013) and Alagendran et al. (2019) found that lemongrass addition enhanced the flavour and aroma of tea while maintaining a smooth mouthfeel. An optimal concentration of 1.0% lemongrass powder yielded a balanced lemony flavour and refreshing aroma, while higher concentrations introduced grassy or bitter notes. Although a slight decline in aroma intensity occurred during storage due to essential oil volatilisation, the tea retained overall acceptability. The characteristic flavour, attributed to citral and citronellal, was complemented by mild sweetness and balanced astringency, contributing to a stable and pleasant sensory experience.

In meat-based products, Sutha and Chandirasekaran (2021) observed that incorporating LEO into chicken nuggets improved flavour and aroma without altering textural properties. A concentration of 0.05% LEO produced a mild lemon-like aroma and balanced spicy flavour, enhancing sensory appeal, whereas higher levels (0.1%) led to excessive pungency and lower acceptability. Texture, juiciness, and mouthfeel remained unchanged, confirming that lemongrass can naturally enhance flavour without compromising product texture.

Applications of lemongrass nanoemulsions have further demonstrated their potential to enhance sensory quality while extending product shelf life. Prakash et al. (2020) found that coating fresh-cut pineapple with a sodium alginate film containing 0.5% lemongrass oil nanoemulsion (citral-based) effectively preserved its natural flavour, aroma, and firmness during 12 days of refrigerated storage. This concentration maintained the fruit's sweetness, color, and crisp texture, whereas higher levels (1%) produced an overpowering citral aroma that reduced consumer acceptability. Similarly, Faheem et al. (2022) reported that nanoemulsified lemongrass oil maintained flavour, aroma, and textural integrity in various foods, including fruits and dairy, while preventing off-flavours or bitterness typically associated with higher concentrations of pure oil. Nanoencapsulation enhanced citral stability and controlled release, sustaining its pleasant citrus aroma and reducing volatilisation. Overall, LEO and its nanoemulsions hold strong potential as natural, multifunctional ingredients for improving the sensory quality, shelf stability, and consumer acceptability of value-added food products.

6.4. Regulatory status, consumer acceptability, and market potential

LEO is widely recognized by regulatory authorities and continues to see rising global demand, supporting its role as a natural preservative and flavoring agent in both food and consumer products. From a safety perspective, the Flavour and Extract Manufacturers Association (FEMA) expert panel has affirmed LEO (FEMA No. 2624) as *generally recognized as safe* (GRAS) for use as a natural flavoring ingredient, following comprehensive toxicological evaluation and constituent profiling (Rosol et al., 2023). Similarly, the European Chemicals Agency (ECHA) recognizes citral as the primary component of LEO and it is safe to use under specified concentrations in food, cosmetics, and household products, though it must be labeled for potential sensitization effects in some consumer goods. This dual recognition in the U.S. and EU enhances consumer confidence and regulatory clarity for manufacturers using LEO in natural formulations.

In terms of consumer perception, lemongrass oil is widely regarded as a clean-label, plant-based ingredient with antimicrobial, aromatic, and therapeutic benefits. Its integration into functional foods, beverages, and aromatherapy aligns with consumer preferences for natural and multifunctional additives. Rosol et al. (2023) noted its long-standing use in global cuisines, teas, and confectionery, with increasing consumption observed in North America and Europe due to the popularity of Southeast Asian culinary trends.

The market potential of LEO is likewise promising. According to report from Global Market Insights in 2025, the global lemongrass oil market is expected to surpass USD 130 million by 2027, driven by rising demand across food, cosmetic, and pharmaceutical sectors. India plays a leading role in global supply. Singh et al. (2022) reported a significant increase in India's LEO exports from 80,280 kg in 1997–2002 to over 2.5 million kg in 2017–2020 generating substantial foreign exchange earnings. Key export destinations include the United States, Canada, Germany, and France. The cultivation of lemongrass also contributes to rural employment, crop diversification, and marginal land utilization, reinforcing its socio-economic importance. In summary, the regulatory recognition, positive consumer perception, and expanding market potential of LEO reflect its growing prominence in food safety applications.

7. Research trends and future development directions

Despite the broad recognition of LEO for its antimicrobial and antifungal properties, key research gaps remain. Most studies have focused on in vitro antimicrobial activity against planktonic cells, with limited application to real food matrices, especially those rich in fats or proteins. These complex systems may reduce the efficacy of LEO due to interactions that limit its bioavailability.

While LEO has shown antibiofilm effects against some pathogens, its specific performance against *B. cereus* biofilms in food environments remains under-investigated. Additionally, there is no existing study evaluating the use of supercritical fluid extraction (SFE)-derived LEO nanoemulsions despite their potential for higher citral purity and thermal stability in targeting *B. cereus* contamination in food.

Mechanistically, although citral disrupts bacterial membranes and impairs metabolic activity, how SFE-derived LEO or its nanoemulsions affect *B. cereus* membrane integrity or biofilm formation remains unclear. Clarifying these pathways is essential for developing effective food preservation strategies.

Further gaps include limited long-term stability data for nanoemulsions, unknown sensory impacts in real food systems, and uncertainties around optimal dosing and safety thresholds for chronic human exposure. Moreover, consumer acceptability of LEO-preserved foods has not been well characterized.

8. Conclusion

LEO, particularly its dominant bioactive compound citral, offers a promising natural strategy for combating *B. cereus*, a persistent threat in the food industry due to its spore resistance and biofilm-forming capability. This review highlights how advancements in extraction techniques significantly influence citral yield and functional quality, while nanoemulsion technology enhances the antimicrobial efficacy and physicochemical stability of LEO in complex food systems.

The incorporation of LEO-based nanoemulsions into food packaging, edible coatings, and sanitation protocols demonstrates considerable potential in extending shelf life, reducing microbial load, and aligning with consumer demand for clean-label, plant-based preservatives. However, further investigations are warranted to evaluate their long-term stability, interactions with food components, and sensory impacts. Additionally, regulatory harmonisation and in vivo toxicological data remain critical for commercial adoption.

In conclusion, citral-rich LEO nanoemulsions represent a scalable and sustainable alternative to synthetic preservatives, with substantial promise in ensuring microbiological safety, particularly against *B. cereus*, across diverse food applications.

CRediT authorship contribution statement

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

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Declaration of competing interest

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Data availability

This study did not generate any data.

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