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Citrus essential oils – Based nano-emulsions: Functional properties and potential applications

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ABSTRACT

Citrus essential oils are natural products with various bioactive properties (e.g., antimicrobial, antioxidant, and antimutagenic activities), that are generally recognized as safe (GRAS) by Food and Drug Administration (FDA) to be used as flavorings and food additives. Nonetheless, due to their high volatility, low solubility in water, low thermal stability, susceptibility to oxidation, and strong flavor, their applications in the food industry are limited. Nanotechnology allows the incorporation of citrus essential oils into nano-emulsion systems, thus protecting them from the deterioration caused by external factors and maintaining or even improving their functional properties. This study aims to summarize the antioxidant, antimicrobial, and antimutagenic effects of the nano-emulsions based on essential oils from citrus peels with emphasis on their mechanisms of action and potential applications in, e.g., foods, pharmaceuticals, and cosmetics.

1. Introduction

Essential oils are aromatic metabolites which naturally derived from different parts of plants (flowers, buds, seeds, leaves, herbs, wood, fruits, and roots) or as by-products of the juice industry (Liew et al., 2020). Their composition is based on a mixture of terpenes, terpenoids, aromatic and aliphatic compounds. The chemical composition depends on the biological origin and purification methods (Yazgan, Ozogul, & Kuley, 2019). The main characteristics of the essential oils are their pleasant flavors and bioactive compounds. The main compounds present in citrus essential oils are limonene and linalool. These flavor compounds are available for the food, pharmaceutical, cosmetics, perfumery, and chemical industries.

Nano-emulsions (NEs) are biphasic mixtures of two immiscible liquids (oil and water) stabilized by a surfactant. Depending on the degree of dispersibility of one liquid in the other, they may be oil-in-water (o/w) or water-in-oil (w/o) nano-emulsions (Liew et al., 2020). Citrus essential oil nano-emulsions (CEO-NEs) are food-friendly additives that replace synthetic ones, to obtain a green food. Citrus essential oils are recognized by FDA as generally regarded as safe (GRAS) for use as a flavoring and additive compound in food. Nano-emulsions can be used easily in drinks and beverages due to their low opacity and sub-cellular dimensions (20–200 nm) (Sampaio et al., 2022; Zahi, Liang, & Yuan, 2015). Using nano-emulsions in the food field solves the problem of degradation of essential oils following exposure to environmental conditions. Due to their bioactivity, CEO-NEs can be successfully used as antioxidant, antimicrobial, therapeutic, and larvicidal agents in many industries (Fig. A1).

Additionally, nano-emulsions can form a barrier against bacteria, fungi, and viruses. This mechanism involves the selective binding of the transparent/semi-transparent particles of the nano-emulsion to the cell wall of prokaryotic cells resulting in its destabilization (Kosker, 2020). This passive mechanism of cell absorption does not affect the eukaryotic cell wall due to its membrane which is complex in structure and shape

Abbreviations: FDA, Food and Drug Administration; NEs, nano-emulsions; CEO, citrus essential oil; CEO-NEs, citrus essential oil nano-emulsion; TaEO, tangrine essential oil; TaEO-NEs, tangerine essential oil nano-emulsions; GEO, grapefruit essential oil; TaEO-NEs, grapefruit essential oil nano-emulsions; LeEO, lemon essential oil; TaEO-NEs, lemon essential oil nano-emulsions; OEO, orange essential oil; OEO-NEs, orange essential oil nano-emulsions; MIC, minimum inhibitory concentration; MBC, minimum bacteriostatic concentration; N/A, not applicable; G^-, Gram negative; G^+, Gram positive; HPVH, High-Pressure Homogenization.

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(Wen et al., 2021). In general, CEOs are incorporated into O/W type nano-emulsions to avoid unwanted volatilization and even out the oil's distribution in the aqueous layer (Severino et al., 2014). Usually, the bacteria cells are located at the solid–liquid interface or in the aqueous phase, thus the presence of a nano-system helps to increase the concentration of bioactive compounds, which would normally destabilize the cell wall.

Unfortunately, pure essential oils are not stable under environmental stress. Water insolubility and volatility are the main factors that limit their use in food and beverages for long periods. Other factors responsible for the stability of lipid compounds are environmental stressors (light, heat, humidity, pH, and oxygen). Thus, the lability of the specific compounds in the essential oils can cause reactions with some components from the matrices in which they are introduced, reducing their antioxidant and antimicrobial activities. It is necessary to nano-encapsulate them for these reasons. When essential oils are nano-encapsulated, they are more effective. The chemical stability, organoleptic properties, and delivery of EO-based products are enhanced by this method (Donsi & Ferrari, 2016). Various strategies exist for the encapsulation of essential oils (EOs) into nano-scale particles, encompassing polymer-based nanocarriers (nano-capsules, nano-gels), lipid-based nanocarriers (liposomes, nano-emulsions, solid lipid nano-particles, self-nano-emulsifying drug delivery systems) and molecular complexes. Nano-emulsions (NEs) have demonstrated significant promise across several domains, particularly in the realms of pharmaceutics, biotechnologies, and the food industries. This is primarily attributed to their ability to enhance the bioavailability of lipophilic substances through the creation of expanded interfacial regions. The long-term prevention of coalescence, sedimentation, and flocculation in droplets is attributed to their tiny dimensions and the presence of Brownian motion, which classifies them as kinetically stable particles, while being regarded disequilibrated systems. Taking into account the aforementioned factors, it can be concluded that NEs are a more appropriate choice for transporting citrus essential oils due to their ability to maintain their physical qualities over extended durations (Oprea et al., 2022).

Therefore, during nano-emulsions bioactivity characterization, not only the physicochemical properties, but also the food and human-friendly behaviour have to be taken into consideration. In order to obtain stable functionalized NEs, which would be ready to be used in food as green additives or in medicine as non-invasive treatments (Ibrahium et al., 2022; Lu et al., 2020) with positive results, a series of tests and optimizations of the compatibility between the type of oil, nano-emulsion and the matrix in which it will be used, must be performed.

In this context, the current review aims to summarize the recent progress (last 10 years) made regarding the bioactivity of CEO-NEs, with a specific focus on the biological effect of CEO-NEs (antioxidant, antibacterial and cytotoxic activities) for different food matrices, supplements, drugs, and cosmetic products.

2. Methodology of the literature review

A comprehensive review of the literature was undertaken using Scopus database (search according to title and abstract), specifically examining the findings obtained on 30th September 2023 (Fig. A1). The search was conducted in two stages: firstly, were used the keywords "essential oils nano-emulsions" and "bioactivity" to retrieve results spanning the years 2013 to 2023. After this, the keywords "citrus essential oils nano-emulsions" and "bioactivity" were used in the search of results from 2013 until 2023. The papers were categorised based on their publication year in order to facilitate organisation and give a chronological framework for the research pertaining to this particular topic matter. As can be seen in Fig. A1, there has been a noticeable attention given to essential oils nano-emulsions in the last ten years, with a particular emphasis on their bioactivity and industrial

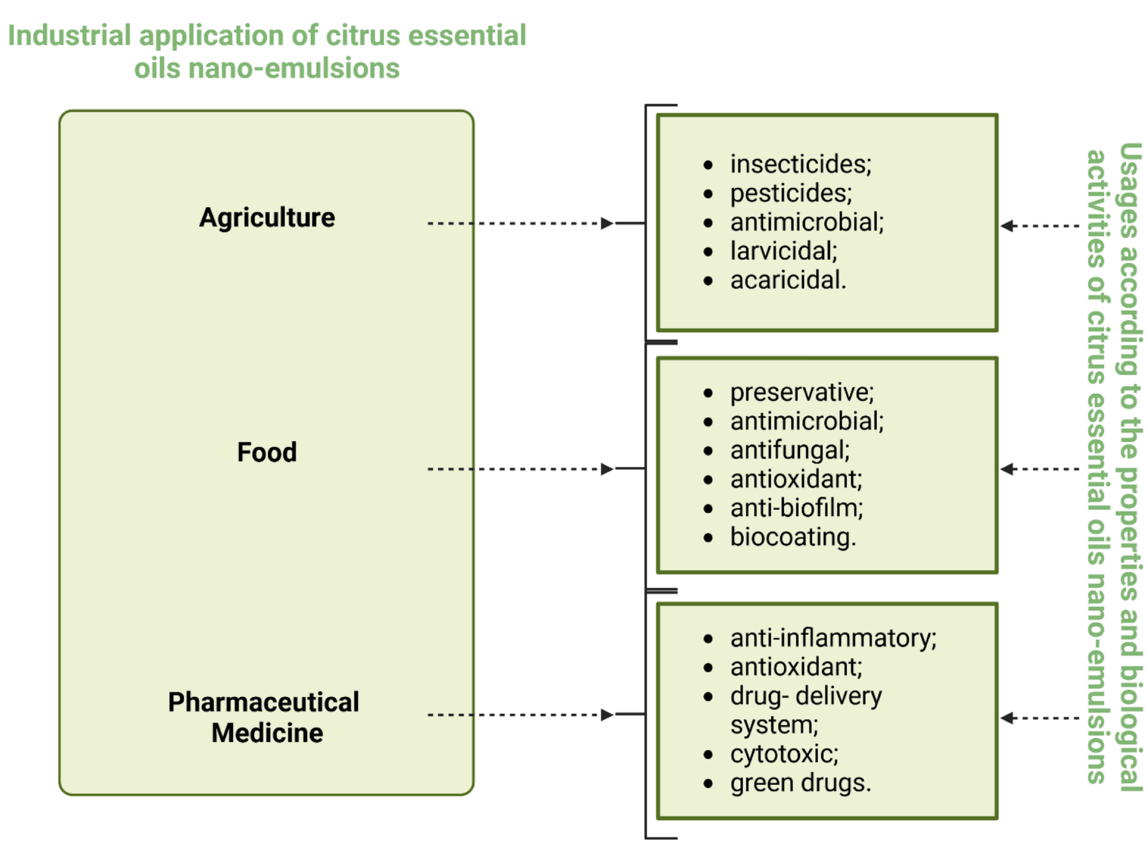

Fig. A1. The industrial application and usages of citrus essential oils nano-emulsions.

applicability. On the other hand, the publications about citrus essential oils nano-emulsions registered a notable increase in 2019 (Fig. A1).

Based on the data survey, it was found that the number of publications about citrus essential oils nano-emulsions is about four times smaller than essential oils nano-emulsion's papers. There are multiple publications on the formulation's methods and industrial applicability of citrus essential oils nano-emulsions and a lack of reviews that summarize specific data related to chemical, microbiological and biological behaviour of citrus essential oils nano-emulsions.

3. Stability of citrus essential oils nano-emulsions

Even if nanoencapsulation is used to increase the stability of the essential oils, the nano-emulsions obtained may have instability during storage due to certain environmental factors, or caused by the internal properties of the emulsions. Physicochemical mechanisms of nano-emulsions instability are flocculation, cremation, sedimentation, coalescence, Ostwald ripening and phase separation (Razola-Diaz, Guerra-Hernandez, Garcia-Villanova, & Verardo, 2021). Flocculation involves the joining of two or more droplets to form an aggregate. Cremation and sedimentation are mechanism in which gravitational separation occurs due the difference in density between the dispersed phase and aqueous phase. Coalescence is the process by which two or more droplets come together to form a larger droplet (Asadinezhad, Khodaiyan, Salami, Hosseini, & Ghanbarzadeh, 2019). Making a comparison between normal emulsion and nano-emulsion, the first is more sensitive to flocculation, coalescence and phase separation, while the last is more susceptible to Ostwald ripening (Walker, Gumus, Decker, & McClements, 2017). Recent studies have examined some of these major factors influencing the instability phenomena and ways of diminishing and keeping them under control. Maswal and Dar (2014) in his study explained the resistance of some nano-emulsions to creaming and flocculation. This resistance to creaming is caused by their Brownian motion which overcomes their low gravitational separation force, while resistance to creaming is caused by steric stabilization. On the other hand, Zhao et al. (2018) points out that the composition and the inherent properties of the essential oils influence the emulsifying properties and instability mechanism of citrus oil emulsion. Zhao et al. (2018) performed a comparison between mandarin and bergamot emulsion, showing that bergamot emulsion is more stable to cremation and coalescence due to their polar and water-insoluble compounds. The small emulsion droplets have a higher local oil solubility than the larger droplets because of the difference in Laplace pressures. This stems from the fact that molecules on the surface of a particle are energetically less stable than the ones in the interior. Ostwald ripening leads to the destabilization of nano-emulsions due to creaming and sedimentation (Maswal & Dar, 2014). Also, the method of preparation is very important for the stability of the formulated nano-emulsion. In the literature, two main preparation methods for oil-in-water nano-emulsions are available; these are formed by low-energy methods or high energy methods, the difference between them is the amount of mechanical energy input (Su & Zhong, 2016).

4. Antioxidant activity of nano-emulsions based on citrus essential oils

It is well known that CEOs have antioxidant capabilities due to their chemical composition. Their high terpene (limonene, linalool, b-pinene) content gives them antioxidant potential (Asadinezhad et al., 2019). Naringenin is a citrus flavanone (preponderantly found in grapefruits) which possesses antioxidant activity, having the capacity to chelate metals, scavenge oxygen free radicals and prevent oxidation of low-density lipoproteins (Khan, Kotta, Ansari, Sharma, & Ali, 2015). Carotenoids are natural pigments that exhibit important antioxidant activities, bringing benefits such as: blocking the action of free radicals on cells, preventing cancer and aging (Maoka, 2020).

The use of NEs as functional systems is a good opportunity for the food industry and for human health to promote natural alternatives to prevent oxidative damage and metabolic disorders caused by reactive oxygen and nitrogen species. In this sense, the use of citrus essential oils is a step toward the evolution of green treatments. Denkova-Kostova et al. (2021) comparatively analyzed the antioxidant behavior of tangerine (TaEO), grapefruit (GEO), lemon (LeEO), and cinnamon essential oils. Based on research, it has been shown that GEO has the best antioxidant activity (87,5%), followed by LEO (86.1%) and finally TaEO (78%), and cinnamon (69.3%). Unfortunately, these results are not high enough to be suitable for use as natural antioxidants in food. This is a good reason for EOs to be encapsulated in nano-emulsions to enhance their antioxidant activity (Denkova-Kostova et al., 2021).

Previous studies (Amjadi, Almasi, Ghadertaj, & Mehryar, 2021; Cenobio-Galindo et al., 2019; Espino-Manzano et al., 2020; Sampaio et al., 2022) have evaluated the antioxidant activity of nano-emulsions (NEs) using the Folin Ciocalteu, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC and FRAP assays. These methods aim to evaluate the NEs antioxidant capacity as a radical scavenger. ABTS has a higher sensibility for essential oils, compared to the DPPH test, reason, why the results obtained for the CEO-NEs following the ABTS analysis are more relevant. The pure essential oils have a low solubility in water, which can minimize their antioxidant activity. However, the essential oil nano-emulsions had excellent solubility in an aqueous system, making them suitable for the efficient delivery of active ingredients, and their large surface area allowed for the rapid penetration of active compounds. Therefore, nano-emulsified essential oils can scavenge radicals more effectively and manifest greater reducing power, enhancing the radicals' scavenging capability and reducing power (Lou et al., 2017).

Several authors (Amjadi et al., 2021; Cenobio-Galindo et al., 2019; Espino-Manzano et al., 2020; Sampaio et al., 2022) have reported in the literature the results of antioxidant activity: Sampaio et al., evaluated the antioxidant activity of lemon balm oil and its nano-emulsions not obtaining satisfactory results by any of the methods (Sampaio et al., 2022), Amjadi et al., obtained good values by DPPH assay for citrus essential oil nano-emulsions incorporated in biofilms (Amjadi et al., 2021), Espino-Manzano et al, carried out nano-emulsions of extract cactus pear fruits and orange oil, incorporated in gelatine films to evaluate the antioxidant activity. Both, the extract of cactus and orange oil have bioactive compounds with antioxidant activity. The film's antioxidant activity was evaluated based on the content of phenols (ABTS and DPPH methods) and flavonoids (Espino-Manzano et al., 2020). Cenobio-Galindo used the orange essential oil NEs as a coating for avocado fruits to improve their properties during storage. Total phenols (Folin Ciocalteau), total flavonoids, and antioxidant activity (DPPH and ABTS) were evaluated. For total phenols and flavonoids analysis, the best results after 60 days were obtained for treatments that contain nano-emulsions diluted with 50 and 25%. In terms of antioxidant activity, the results obtained using the DPPH method are similar to those obtained by ABTS. The highest antioxidant activity during the 60 days, was owned by 25% diluted nano-emulsions, while the lowest was owned by undiluted ones. The conclusion of the study was that dilute NEs form much more stable coatings than pure ones. This can be attributed to a mechanism of stress produced by nano-emulsions on the fruit, which can influence the production of bioactive compounds (Cenobio-Galindo et al., 2019).

On the other hand, Ucar et al. has presented an oxidation problem in aquaculture due to the fatty acids from fish meat. The use of citrus essential oils nano-emulsions as a natural preservative is a good treatment for problems caused by oxidation. During the research, the content of polyphenols that possess antioxidant activity due to their ability to bind to free radicals was analyzed. Treatment of trout samples with citrus nano-emulsions (CENEs) significantly reduced the oxidation of fatty acids, creating a barrier between lipid compounds and oxygen. Evaluating the results could be observed that CENEs do not have a significant impact on saturated fatty acids presenting relatively small quantitative reports of them in the treated fish. The best delay of the oxidative phenomenon is registered in the case of nano-emulsions containing grapefruit essential oil, which can be confirmed by its large amount of polyphenols. On the other hand, the CENEs have a positive effect on monosaturated fatty acids ensuring a minimum level of oxidation during storage. The highest monounsaturated fatty acids (MUFA) amount was observed for OEONEs, while the lowest was observed for CEONEs. In the case of polyunsaturated fatty acids, the highest ratio was observed for samples treated with mandarin essential oil nano-emulsions, while the lowest was observed for grapefruit and lemon essential oil nano-emulsions. Due to their antioxidant activity, CEONEs inhibit lipid oxidation in the muscle tissue of fish (Ucar, 2020).

According to the results obtained by Lou using the DPPH method, for both pure essential oils and CEONEs, scavenging activity is obviously better for nano-emulsions than for pure EOs. It has been observed an increase from 30.5% (pure essential oil) to 51.6% (nano-emulsionated essential oil) when a concentration of 0.12 mg/ml EO has been used. For an essential oil concentration four times higher, an antioxidant activity boost was observed (from 44.3% for CEO to 72.4% for CEONEs). This evolution is justified because a higher concentration of essential oil implies a higher concentration of bioactive compounds, which are responsible for the antioxidant activity (Lou et al., 2017).

Walker et al. used lemon oil as fish oil-in-water nano-emulsion. Lemon oil systems present oxidative stability due to the presence of natural antioxidants. The level of oxidation was evaluated using the Folin Ciocalteau method to determine the total phenolic content (Walker et al., 2017).

5. Antibacterial activity of nano-emulsions based on citrus essential oils

Previous studies have focused on studying the antimicrobial activity of citrus essential oils nano-emulsions. This interest has increased due to health-beneficial properties (bactericidal, antiseptic, anti-inflammatory, positive effect on the nervous system, emotional and mental state, stimulating tissue regeneration, and strengthening the immune system) (Azmy et al., 2021; Denkova-Kostova et al., 2021). Also, the nanometric dimensions which facilitate the transport of molecules across the membranes and improve the reactivity of bioactive compounds have an important role. For the preservation of antimicrobial activity and antioxidant properties, it is recommended to use essential oils incorporated in nano-emulsions and not in a pure state because environmental factors can attack their vulnerable properties. Nevertheless, literature shows that not always CEO-NEs have better antimicrobial activity than pure oils.

Lemon essential oil is used in medicine, cosmetics, and the food industry as a flavor additive. This has a role in blood pressure regulation, respiratory problems, rheumatism, and kidney stones. Also, it has antimicrobial and antitumoral activity. Tangerine is used in the food, cosmetic, and pharmacy industries. This EO has antimicrobial, antioxidant, antiseptic, circulatory, and tonic properties. Grapefruit essential oil is a healing agent with antioxidant benefits. This has a role in cancer prevention and strengthens the immune system. Hydrodistillation-derived grapefruit essential oil has an antibacterial and antifungal activity similar to ciprofloxacin (Denkova-Kostova et al., 2021).

Gram-positive and Gram-negative microorganisms differ in the structure of the cell wall, which determines the positive or negative response to the treatment with essential oils/nano-emulsions of essential oils. Gram-positive bacteria have a thick peptidoglycan layer which facilitates the transport of lipophilic molecules along the membrane. On the other hand, gram-negative bacteria have a double membrane and the outer one contains a lipopolysaccharide layer, which makes it difficult to transport bioactive compounds across the membrane (Donsi, 2018). The mechanism of EOs/EONEs transport in the case of Gram-positive membranes can be justified by either altering the integrity of the phospholipid bilayer of the cell membrane or by interfering with the transport protein present in it (Fig. A2). For Gram-negative membranes, the porin proteins present on the cell wall, act as a hydrophilic transmembrane channel and allow the passage of nano-emulsion droplets with a hydrophilic surface (Fig. A3). After treating the prokaryotic cell with essential oils, its membrane degrades, a phenomenon that leads to cell death (Li, Cai, Liu, & Sun, 2018). It is good to know that NEs do not produce the lyse of eukaryotic cells (Kosker, 2020). According to the literature reports, Gram-positive bacteria are more sensitive to nano-emulsions than Gram-negative ones (Allam, Ramadan, Ismail, & Hebeish, 2022).

Foods and vegetables are prone to yeast and pathogenic contamination at any point in the technological flow. In the food industry, both Gram-negative and Gram-positive bacteria can be present: *Salmonella entiritidis, Escherichia coli, Listeria monocytogenes, Listeria innocua* and *Staphylococcus spp*. The effect of nano-emulsions on bacterial microorganisms depends on EOs compounds, the emulsion size, the matrix where the CEO-NEs are used, and the type of microbial strain tested.

The physicochemical properties of nano-emulsions are directly proportional to their antibacterial activity. The droplet size and electric charge of nano-emulsions influenced the transport along the membranes (Donsi, 2018). On the other hand, the surfactant type or ripening inhibitor used for nano-emulsion preparation has an important role in evaluating antimicrobial activity (Prakash, Baskaran, Paramasivam, & Vadivel, 2018). For example, NEs that contain in their structure whey isolate protein (WPI) are better antifungal agents than those which include Tween 80 (Ribes et al., 2017).

Due to their transparent or slightly turbid appearance, they can be used in food products. Literature reports showed that there must be compatibility between the essential oil and the food matrix used, to avoid an unwanted sensory nuance.

According to the literature studies, the EOs are incorporated in the coating matrix for increasing the shelf life and controlling the transport between the outer and inner environment of the protected membrane. Sessa et al. applied modified chitosan containing lemon EOs nano-emulsion on the rucola leaf and observed an increase in shelf life of up to 7 days compared to the control samples. The efficacy of the nano-emulsion-based coatings was found to be higher than the individual components (Sessa, Ferrari, & Donsì, 2015).

Essential oil nano-emulsions have been tested in vitro against different microorganisms ranging from bacterial cells to saprophytic test microorganisms (Li et al., 2018), as shown in Table 1 (antibacterial activity) and Table 2 (antifungal activity: yeasts (Sugumar, Singh, Mukherjee, & Chandrasekaran, 2016) and molds (Guo, Mao, Li, & Zhou, 2023), which summarize the main investigations reported to date, classified in terms of species, active components, amount of essential oil, and impact of nano-emulsions.

Based on the literature reports, the CEO-NEs antimicrobial activity was studied by three methods: minimum inhibitory concentration (MIC), minimum bacteriostatic concentration (MBC), and disc-diffusion method (zone inhibition-ZI). Antifungal activity was tested by MIC and mycelial growth essay (Li et al., 2018).

D-limonene (Lu, Zhang, & Huang, 2014) is one of the most common compounds in CEO, widely used in cosmetics and food industries due to its antimicrobial, antioxidant, chemo-preventive, anticarcinogen, and antidiabetic properties. According to the literature, limonene affects inhibiting the growth of pathogenic and spoilage bacteria (Liew et al., 2020). Unfortunately, once exposed to environmental conditions these properties diminish so it is necessary to nano-encapsulate D-limonene. Zahi et al., chose an organogel-based nano-emulsion to improve the antimicrobial activity of D-limonene. He studied the minimum inhibitory concentrations making a comparison between free D-limonene and D-limonene organogel-based nano-emulsions. As shown in Table 2, free D-limonene shows the most promising bacteriostatic effect for *Saccharomyces cerevisiae* (MIC 0.5 μg/mL), while D-limonene organogel-based NE is susceptible to *Staphylococcus aureus* (MIC 0.125 μg/mL). Looking

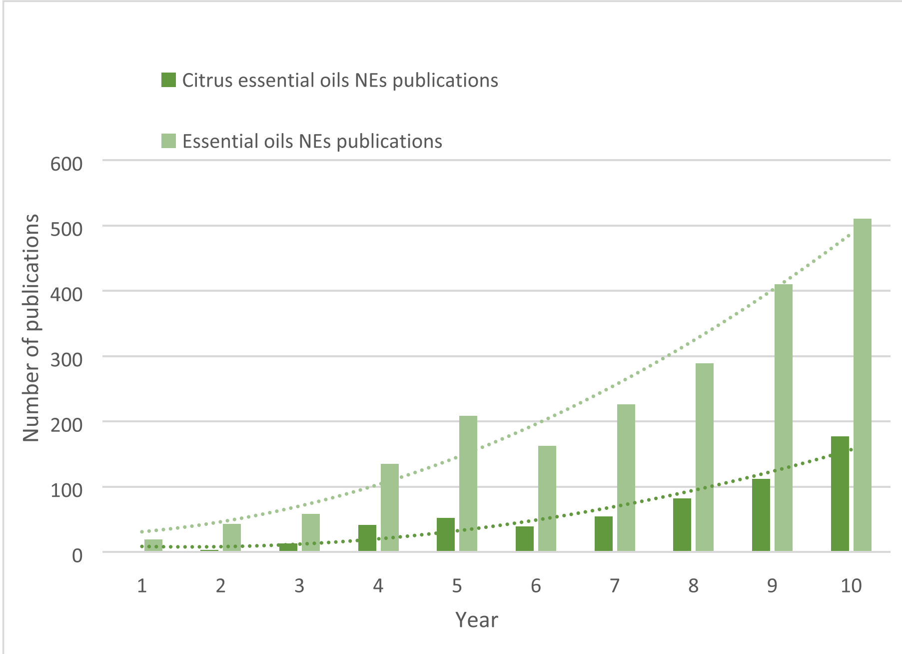

Fig. A2. The trend of publications concerning essential oils nano-emulsions, citrus essential oils nano-emulsions and citrus essential oil's compounds nano-emulsions.

at the whole results for free and protected D-limonene antimicrobial activity, an improvement in the potential for the elimination of microorganisms can be observed. This is also confirmed by the SEM analysis where it can be seen the destruction of the prokaryotic membrane for D-limonene organo-gel-based nano-emulsions (Zahi et al., 2015).

Finger citron essential oil (FCEO) loaded nano-emulsions hurt pathogenic organisms. Nano-emulsions showed better activity against *E. coli, Bacillus subtilis* and *S. aureus* than free essential oil which confirms the potentiation of antimicrobial properties through encapsulation. On the other hand, FCEO-NEs have lower antifungal activity compared with free essential oils which have a strong fungitoxic activity (MIC concentrations and ZI concentrations for *A. niger* and *P. citrinum* are 30 μL/mL, 27.8%, respectively 20 μL/mL and 14.8%) (Li et al., 2018). This has also been observed by Ribes et al., in his study about lemon and bergamot CEO-NEs. She puts this phenomenon based on one main compound of citrus oils assuming that LeEO and BEO tend to dissolve in the aqueous phase at lower concentrations, the driving forces of EOs release in the aqueous phase are reduced, which limits the antimicrobial activity (Ribes et al., 2017). Some authors have chosen to enrich nano-emulsions with nanoparticles of precious metals to improve antimicrobial activity. Van Dat et al., observed a considerable improvement in the antimicrobial activity of orange essential oil nano-emulsions when combined with silver nanoparticles (Dat et al., 2020).

The development of biodegradable active packaging enriched with CEONEs is a good way to improve the shelf life and the qualitative properties of products in the food industry. Amjady et al., have evaluated in their study the properties of whey protein isolate-based films incorporated with nano-emulsions of orange essential oil. After evaluating the antimicrobial activity, it was proved that the essential oil increased the antimicrobial capacity of the films. This is based on the chemical composition of the lipid phase (limonene- the main compound) and the nanometric size of particles (Amjadi et al., 2021). In another study, Radi et al. also used orange essential oil nano-emulsions, but this time incorporated in pectin-based coating for extending the shelf life of fresh-cut oranges. The findings of the study showed that nano-emulsions are more suitable against pathogens than controls or microemulsions. In addition, microorganisms are more susceptible to nano-emulsion treatment than yeasts and molds (Radi, Akhavan-Darabi, Akhavan, & Amiri, 2018). Both Amjady and Radi, were chosen to evaluate the antimicrobial effect, total plate, and fungal counts of coated structures. Das et al., also incorporated orange essential oil in an edible coating for extending the quality of tomatoes. In this study, sodium-alginate was used as a matrix in edible coating formulation. Sodium alginate is a polysaccharide that possesses independent antimicrobial activity, which helps to fulfill the purpose of the research. MIC, MBC, and disc diffusion methods were used to analyze the ability of coatings to prevent the development of pathogens in food matrices (Das, Vishakha, Banerjee, Mondal, & Ganguli, 2020). Lou Z. and collaborators used Citrus medica nano-emulsions as a protective cover on tofu, against *S. aureus*. According to the results, pure essential oil presents a lower inhibitive effect than nano-emulsions, which demonstrates that EONEs enhance the bacterial efficiency (Lou et al., 2017).

Many previous studies evaluated the antimicrobial and antifungal activity of CEO-NE against pathogens and fish spoilage which is caused by Gram-negative psychrophilic bacteria. Durmus et al., used orange, mandarin, grapefruit, and lemon nano-emulsions to extend the shelf life of rainbow trout. The analysis concluded that the best effect was given by mandarin and grapefruit nano-emulsions. The shelf life of the control group was 10 days, for the Tween group 12 days, 14 days for the orange and lemon groups, and 16 days for the mandarin and grapefruit groups. Mandarin and grapefruit essential oils slowed down the growth of the microorganisms (Durmus, 2020). Another study reported the presence in the fish meat of some biogenic amines which are forming during storage and indicate the fish tissue quality. The effects of citrus nano-emulsions (orange, lemon, mandarin, and grapefruit) for the prevention of putrescine, cadaverine, spermidine, spermine, serotonin, tyramine, dopamine, and agmatine were tested. The efficiency of nano-emulsions has been reported according to the quality index (QI) which presented favorable results for lemon and grapefruit nano-emulsions. The treatment using lemon and grapefruit essential oils was effective on putrescine and histamine formation for 16 days (Kosker, 2020). Ozogul et al., evaluated too the antimicrobial effect of grapefruit peel essential oil and its nano-emulsions on fish spoilage bacteria and food pathogens. MIC, MBC, and paper disc diffusion methods were used and their results described the high bacteriostatic effect of pure CEOs and of CEONES (only if the amount of oil is increased) against *Salmonella*

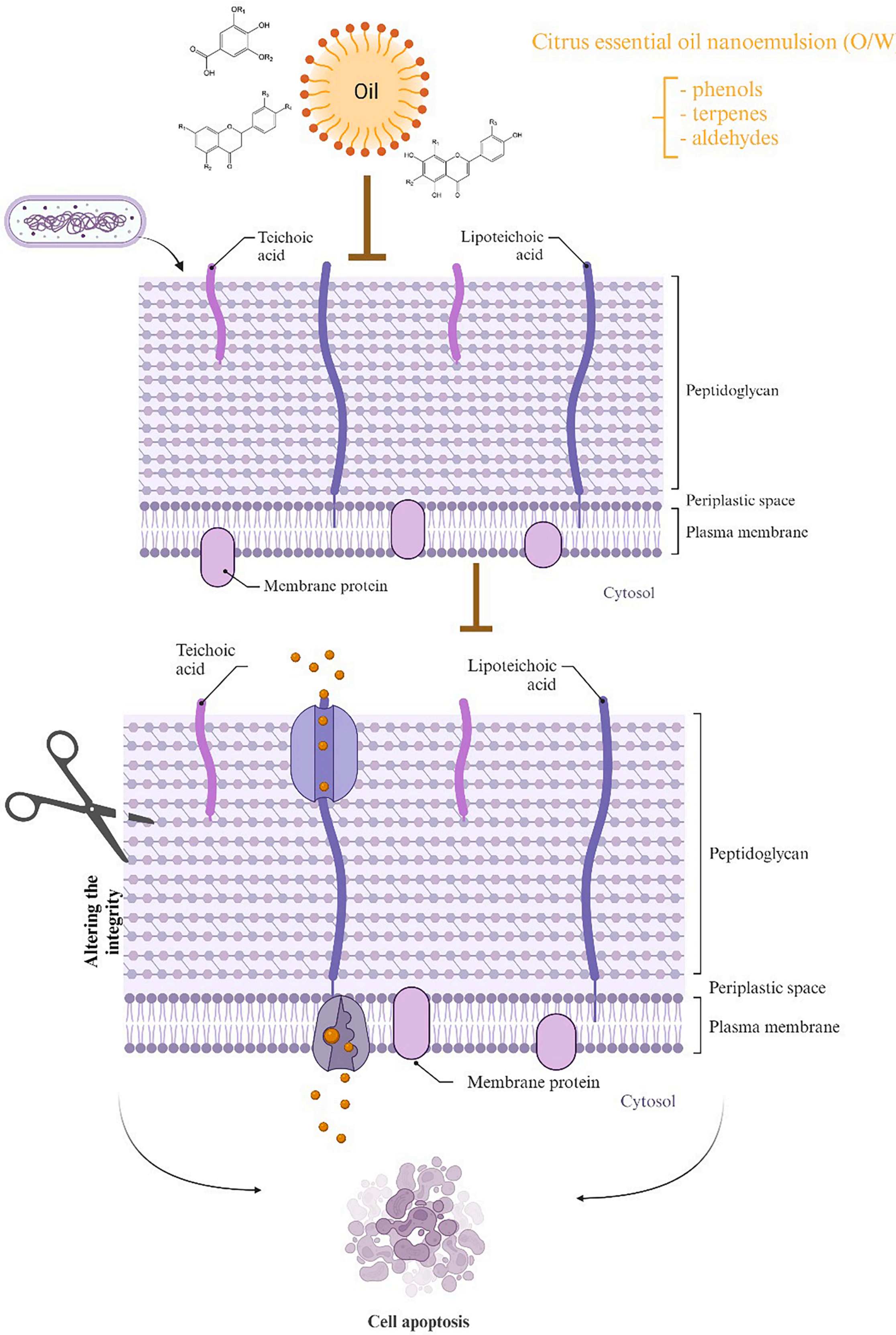

Fig. A3. Effect of citrus essential oils against gram-positive bacteria.

Table 1
Antimicrobial activity of citrus essential oils-based emulsions against pathogenic bacteria.

Antimicrobial activity Microbial strain	*Classification of microbial strain*	*Essential oil/ bioactive compound from EO*	*Amount of essential oil*	*Emulsifier*	*Emulsification technique*	*Impact of nano-emulsions on the bacteria*			*Reference*
						MIC	*MBC*	*Disc Diffusion/ Mycelial growth assay/ bacteria numbers*	
Escherichia coli	*G* $^{-}$	*Lemon*	*100%*	*N/A*	*N/A*	*600 ppm*		*9.67 mm*	(Denkova-Kostova et al., 2021)
Salmonella abony	*G* $^{-}$					*60 ppm*		*10.17 mm*	
Staphylococcus aureus	*G* $^{+}$					*60 ppm*		*14.50 mm*	
Pseudomonas aeroginosa	*G* $^{-}$					*600 ppm*		*10 mm*	
Candida albicans	*N/A*					*6 ppm*		*21 mm*	
Escherichia coli	*G* $^{-}$	*Tangerine zest*				*600 ppm*		*9 mm*	
Salmonella abony	*G* $^{-}$					*600 ppm*		*9 mm*	
Staphylococcus aureus	*G* $^{+}$					*6 ppm*		*20.33 mm*	
Pseudomonas aeroginosa	*G* $^{-}$					*N/A*		*N/A*	
Candida albicans	*N/A*					*60 ppm*		*10.17 mm*	
Escherichia coli	*G* $^{-}$	*Grapefruit*				*600 ppm*		*30.33 mm*	
Salmonella abony	*G* $^{-}$					*600 ppm*		*28.33 mm*	
Staphylococcus aureus	*G* $^{+}$					*6 ppm*		*30.17 mm*	
Pseudomonas aeroginosa	*G* $^{-}$					*600 ppm*		*25.33 mm*	
Candida albicans	*N/A*					*6 ppm*		*30.33 mm*	
Escherichia coli	*G* $^{-}$	*D- limonene*	*100% (free* D-*limonene) 4% (*D-*limonene organogel-based*	*Tween 80*	HPVH	*N/A*	*N/A*	*N/A*	(Abd El-Hack et al. (2022))
Staphylococcus aureus	*G* $^{+}$					*N/A*	*N/A*	*N/A*	
Bacillus subtilis	*G* $^{+}$					*N/A*	*N/A*	*N/A*	
Staphylococcus aureus	*G* $^{+}$	*Citral*	*10%*	*Span 85 + Brij 97*	Ultrasonication	*N/A*	*N/A*	*19.2 mm*	(Lu et al., 2018)
Escherichia coli	*G* $^{-}$					*N/A*	*N/A*	*9.4 mm*	
Pseudomonas aeroginosa	*G* $^{-}$					*N/A*	*N/A*	*6.2 mm*	
Enterococcus faecalis	*G* $^{-}$					*N/A*	*N/A*	*10.2 mm*	
Salmonella Typhimurium	*G* $^{-}$					*N/A*	*N/A*	*2.0 mm*	
Listeria monocytogenes	*G* $^{+}$					*N/A*	*N/A*	*14.4 mm*	
Escherichia coli	*G* $^{-}$	*Finger citron*	*1%*	*Cremophor EL*	Spontaneous emulsification	*N/A*		*N/A*	(Li et al., 2018)
Bacillus subtilis	*G* $^{+}$					*N/A*			
Staphylococcus aureus	*G* $^{+}$					*N/A*			
Listeria monocytogenes	*G* $^{+}$	*Orange*	*4%*	*Tween 80*	Ultrasonication	*N/A*		*13 mm*	(Amjadi et al., 2021)
Escherichia coli	*G* $^{-}$					*N/A*		*8.67 mm*	
Salmonella Typhimurium	*G* $^{-}$					*N/A*		*11.67 mm*	
Pseudomonas aeroginosa	*G* $^{-}$					*N/A*		*6.33 mm*	
Mesophilic bacteria	*G* $^{-}$	*Orange*	*1%*	*Tween 80*	Ultrasonication	*N/A*		*N/A*	(Radi et al., 2018)
Salmonella Typhimurium	*G* $^{-}$	*Orange*	*2%*	*Tween 80*	Ultrasonication	*N/A*	*N/A*	*14 mm*	(Das et al., 2020)
Listeria monocytogenes	*G* $^{+}$					*N/A*	*N/A*	*13 mm*	

(continued on next page)

Table 1 (*continued*)

Antimicrobial activity Microbial strain	*Classification of microbial strain*	*Essential oil/ bioactive compound from EO*	*Amount of essential oil*	*Emulsifier*	*Emulsification technique*	*Impact of nano-emulsions on the bacteria*			*Reference*
						MIC	*MBC*	*Disc Diffusion/ Mycelial growth assay/ bacteria numbers*	
Mesophilic bacteria	G $^{-}$	*Mandarin*	4%	*Tween 80*	Ultrasonication	*N/A*	*N/A*	*N/A*	(Durmus, 2020)
		Grapefruit				*N/A*	*N/A*	*N/A*	
Psychrophilic bacteria	G $^{-}$	*Mandarin*				*N/A*	*N/A*	*N/A*	
		Grapefruit				*N/A*	*N/A*	*N/A*	
Enterobacteriaceae	G $^{-}$	*Mandarin*				*N/A*	*N/A*	*N/A*	
		Grapefruit				*N/A*	*N/A*	*N/A*	
Staphylococcus aureus	G $^{+}$	*Grapefruit*	100%	*Tween 80*	Ultrasonication	*N/A*	*N/A*	*10.25 mm 7.88 mm*	(Ozogul et al., 2021)
Enterococcus faecalis	G $^{+}$					*N/A*	*N/A*	*6.50 mm 0 mm*	
Klebsiella pneumonia	G $^{-}$					*N/A*	*N/A*	*7.50 mm 6.00 mm*	
Salmonella Paratyphi A	G $^{-}$					*N/A*	*N/A*	*10.50 mm 6.25 mm*	
Staphylococcus aureus	G $^{+}$	*Lemon*	100% and 10%	*Tween 80*	Ultrasonication	*N/A*	*N/A*	*16.63 11.63 16.25 mm*	(Yazgan et al., 2019)
Klebsiella pneumonia	G $^{+}$					*N/A*	*N/A*	*14.38 19.75 11.50 mm*	
Enterococcus faecalis	G $^{-}$					*N/A*	*N/A*	*24.25 19 10.50 mm*	
Salmonella Paratyphi A	G $^{-}$					*N/A*	*N/A*	*17.88 13.38 24.25 mm*	
Pseudomonas aeroginosa	G $^{-}$	*Lime*	1%	*Tween 80*	Ultrasonication	*N/A*	*N/A*	*15 mm*	(Thomas et al., 2014)
Escherichia coli	G $^{-}$	*Bergamot*	2%	*Tween 20*	HPVH	*N/A*	*N/A*	*N/A*	(Severino et al., 2015)
		Mandarin				*N/A*	*N/A*	*N/A*	
		Lemon				*N/A*	*N/A*	*N/A*	
Salmonella Typhimurium	G $^{-}$	*Bergamot*				*N/A*	*N/A*	*N/A*	
		Mandarin				*N/A*	*N/A*	*N/A*	
		Lemon				*N/A*	*N/A*	*N/A*	
Listeria innocua	G $^{+}$	*Mandarin*	2%	*Tween 20*	HPVH	*N/A*	*N/A*	*N/A*	(Severino et al., 2014)
						N/A	*N/A*	*N/A*	
						N/A	*N/A*	*N/A*	
Listeria innocua	G $^{+}$	*Mandarin*	2%	*Tween 20*	HPVH	*N/A*	*N/A*		(Donsi, 2018)
						N/A	*N/A*	*N/A*	(Donsi, 2018)

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Table 1 (*continued*)

Antimicrobial activity Microbial strain	*Classification of microbial strain*	*Essential oil/ bioactive compound from EO*	*Amount of essential oil*	*Emulsifier*	*Emulsification technique*	*Impact of nano-emulsions on the bacteria*			*Reference*
						MIC	*MBC*	*Disc Diffusion/ Mycelial growth assay/ bacteria numbers*	
Escherichia coli	*G* –	*Lemon myrtle*	*N/A*	*Tween 80*	Emulsion phase inversion	*N/A*	*N/A*	*0.97 0.97 0.90 mm*	(Sampaio et al., 2022)
Staphylococcus aureus	*G* $^{+}$					*N/A*	*N/A*	*0.90 0.90 1.35 mm*	
Escherichia coli	*G* –					*N/A*	*N/A*	*7.60 mm*	
Salmonella spp	*G* –					*N/A*	*N/A*	*7.28 mm*	
Staphylococcus aureus	*G* $^{+}$	*Key lime*				*N/A*	*N/A*	*8.34 mm*	
Escherichia coli	*G* –		*5%*	*Tween 80*	Spontaneous emulsification	*N/A*	*N/A*	*7.65 mm*	(Liew et al., 2020)
Salmonella spp	*G* –					*N/A*	*N/A*	*7.29 mm*	
Staphylococcus aureus	*G* $^{+}$	*Kaffir lime*				*N/A*	*N/A*	*7.71 mm*	
Escherichia coli	*G* –					*N/A*	*N/A*	*8.34 mm*	
Salmonella spp	*G* –					*N/A*	*N/A*	*7.71 mm*	
Staphylococcus aureus	*G* +	*Calamansi lime*				*N/A*	*N/A*	*9.98 mm*	
Escherichia coli	*G* –	*Orange*	*10%*	*Tween 80*	Emulsion phase inversion	*N/A*	*N/A*	*N/A*	(Bento et al., 2020)
Escherichia coli	*G* $^{-}$			*Glycerol monooleate + Tween 20*		*N/A*	*N/A*	*N/A*	
Lactobacillus delbruecki	*G* +	*Bergamot*	*2%*		HPVH	*N/A*	*N/A*	*N/A*	(Marchese et al., 2020)
Citrobacter diversus Proteus vulgaris Escherichia coli	*G* $^{-}$	*Mandarin*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	(Khalaf et al., 2022)

Gram negative – G $^{-}$.
Gram negative – G $^{+}$.
N/A- not applicable.

Parathypi A and *P. luteola*. The bactericidal effect was lower and grapefruit essential oil could be considered susceptible just for *P. luteola*, while grapefruit oil-based NE does not show a bactericidal effect for any microorganism. This study is a confirmation of the hypothesis that not always CEO-NEs have better antimicrobial activity than pure oils, some improvements should be applied (Ozogul, Ozogul, & Kulawik, 2021). The same author used in another study lemon essential oil-based nano-emulsions against the same food pathogens and fish spoilage bacteria as in the previous study (Yazgan et al., 2019). Comparing the results of the two studies following MIC, MBC, and disk diffusion analyses we can conclude that LeO and LeO-NEs are much more likely to be used against fish pathogens and bacteria spoilage than GEO and its NEs. Unlike the previous study which used the citrus essential oils nano-emulsions for the antifungal activity, Amr Farouk et al. obtained a better response for NEs than for pure essential oil (Mansour, Hathout, Amer, Hussain, & Fouzy, 2022). For this reason, to ensure consumer health, the use of nano-emulsions prepared from citrus essential oils would be an option to prevent toxic compounds in seafood.

Sometimes, to ensure an efficient treatment and a strong preservation capacity for the pathogens, EONEs are combined with some physical processes; this strategy is called hurdle technology (Prakash et al., 2018). Severino et al. analyzed the behavior of individual essential oils coating or in combination with physical processes (modified atmosphere packaging and gamma irradiation) against *Salmonella Tiphymuriumand* and *E. coli* inoculated on green beans. Carvacol, mandarin, bergamot, and lemon were tested. Unfortunately, CEOs presented high MIC values against *Salmonella Tiphymuriumand* and *Escherichia coli* and are not used in the development of coating systems. However, making a comparison only between the nano-emulsions of citrus oils, bergamot oil-loaded nano-emulsions have the best activity against *S. Tiphymuriumand* (MIC = 2) (Severino et al., 2015). In a different study, the same authors used another three physical processes to achieve bio-preservation (UV-C, ozonated water, gamma irradiation) in combination with chitosan coating containing mandarin oil against *Listeria innocua* inoculated on green beans. The combination between gamma radiation and coating showed the best anti-Listeria response, while the combination of UV-C coating showed no antimicrobial improvement (Severino et al., 2014). Donsi and collaborators used mandarin coating

Table 2
Antifungal activity of citrus essential oils-based emulsions against yeasts and molds.

Antifungal activity Yeast and Molds	Essential oil/ bioactive compound from EO	Amount of essential oil	Emulsifier	*Emulsification technique*	Impact of nano-emulsions on the molds			References
					MIC	MBC	Disc diffusion/ Mycelial growth assay	
Bacillus subtilis					60 ppm	N/A	13.33 mm	
Saccharomyces cerevisiae					6 ppm	N/A	27.33 mm	
Aspergillus Niger					600 ppm	N/A	9 mm	
Penicillium chrysogenum					60 ppm	N/A	19 mm	
Fusarium moniliforme					60 ppm	N/A	14.67 mm	
Aspergillus flavus	Lemon	100%	N/A	N/A	6 ppm	N/A	24.17 mm	(Denkova-Kostova et al., 2021)
Bacillus subtilis					60 ppm	N/A	15.50 mm	
Saccharomyces cerevisiae					6 ppm	N/A	25.17 mm	
Aspergillus Niger					600 ppm	N/A	9 mm	
Penicillium chrysogenum					6 ppm	N/A	20.17 mm	
Fusarium moniliforme	Tangerine zest				60 ppm	N/A	17 mm	
Aspergillus Flavus					6 ppm	N/A	22 mm	
Bacillus subtilis					6 ppm	N/A	20.33 mm	
Saccharomyces cerevisiae	Grapefruit				6 ppm	N/A	28 mm	
Aspergillus Niger					6 ppm	N/A	20.17 mm	
Penicillium chrysogenum					60 ppm	N/A	13.67 mm	
Fusarium moniliforme					6 ppm	N/A	24.67 mm	
Aspergillus flavus					6 ppm	N/A	25.67 mm	
Saccharomyces cerevisiae	D-limonene	100%) 4% (D-limonene organogel-based	Tween 80	HPVH	N/A	N/A	N/A	(Mohamed Reda Zahi, Liang, & Yuan, 2014)
Penicillium citrinum	Finger				N/A	N/A	N/A	
Aspergillus Niger	citron	1%	Cremophor EL	Spontaneous emulsification	N/A	N/A	N/A	(Li et al., 2018)
Yeasts and molds	Orange	1%	Tween 80	Ultrasonication	N/A	N/A	N/A	(Radi et al., 2018)
Saccharomyces cerevisiae	Orange	6%	Tween 80	Ultrasonication	N/A	N/A	N/A	(Sugumar et al., 2016)
Photobacterium damselae					N/A	N/A	10.75 mm 15.66 mm	
Enterococcus faecalis					N/A	N/A	0	
Vibrio vulnificus					N/A	N/A	6.75 mm	(Ozogul et al., 2021)
Proteus mirabilis					N/A	N/A	0	
Serratia liquefaciens	Grapefruit	100%	Tween 80	Ultrasonication	N/A	N/A	8.38 mm	
Pseudomonas luteola					N/A	N/A	14.24 mm 5.00 mm	
Photobacterium damselae					N/A	N/A	19.25 25 17.75 mm	

(*continued on next page*)

Table 2 (*continued*)

Antifungal activity Yeast and Molds	Essential oil/ bioactive compound from EO	Amount of essential oil	Emulsifier	*Emulsification technique*	Impact of nano-emulsions on the molds			References
					MIC	MBC	Disc diffusion/ Mycelial growth assay	
Enterococcus faecalis				Ultrasonication	N/A	N/A	17.75 8.75 24.75 mm	
Vibrio vulnificus					N/A	N/A	16.75 15.38 15 mm	
Proteus mirabilis					N/A	N/A	12.38 18.38 15 mm	(Yazgan et al., 2019)
Serratia liquefaciens	Lemon	100% and	Tween 80		N/A	N/A	14.75 22 26.25 mm	
Pseudomonas luteola		10%			N/A	N/A	17.25 20.50 17.75 mm	
Aspergillus niger	Lemon essential oil Bergamot essential oil	100%	Tween 80 WPI	HPVH	N/A N/A	N/A N/A	N/A N/A	(Ribes et al., 2017)
Saccharomyces cerevisiae	Bergamot	2%	Glycerol monooleate + T20	HPVH	N/A	N/A	N/A	(Marchese et al., 2020)
Candida glabrata	Mandarin	N/A	N/A	N/A	N/A	N/A	N/A	(Khalaf et al., 2022)
Candida tropicalis								
Aspergillus flavus *Aspergillus niger* *Aspergillus ochraceus*					N/A N/A N/A	N/A N/A N/A	17.5 ± 0.71 10.5 ± 2.12 11.0 ± 0.00	
Fusarium spp. *Penicillium* spp.	Sweet Orange	5%	Polysorbate 20	Ultrasonication	N/A N/A	N/A N/A	N/A N/A	(Mansour et al., 2022)

N/A-not applicable.

EONEs associated with different treatments against *Listeria innocua* on green beans. High hydrostatic pressure (HHP) and pulsed light (PL) are the non-thermal treatments used. The samples treated with PL and bioactive coating don't show any synergistic effect against bacteria, while the combination between HHP and bioactive coating shows (Donsi, 2018; Donsi et al., 2015).

As we mentioned, due to their small size, nano-emulsions are often used in beverages to increase their shelf life. Bento et al., evaluated the antimicrobial activity of orange essential oil and its chitosan-loaded nano-emulsions combinate with mild heat in orange and apple juices. It is necessary to combine essential oils with an extra treatment to obtain a synergistic effect against microorganisms in food. According to the analysis results, the antimicrobial activity is influenced by the matrix type (in our case fruit type: orange or apple). The best antimicrobial activity was observed in apple juice when nano-emulsions of OEO with chitosan and mild heat are used, while orange juice accepts better mild heat combined with suspension of essential oil. These differences in compatibility are due to the chemical composition of juices and the interaction of compounds in the composition of juices and nano-emulsions (Bento et al., 2020).

The CEOs nano-emulsions showed great potential to be incorporated into food products and beverages as a flavoring and antimicrobial agent (CEOs used have properties against a wide range of pathogenic and non-pathogenic microorganisms found in foods) (Khalaf, Ragab, Mansour, Elshamy, & El-Gendy, 2022; Liu, Li, & Chen, 2021a).

6. Cytotoxicity of nano-emulsions based on citrus essential oils

Cancer is one of the leading causes of early death among mankind. This disease represents a chaotic proliferation of the cells, practically a normal cell is transformed into an atypical one (Fig. A4). This process is due to the interaction between genetic and external factors (Alkhatib, Alharbi, & Mahassni, 2017). Generally, the treatment of cancer consists of surgery, radiotherapy, or chemotherapy. Unfortunately, these treatments come with several side effects that affect the body's functions, which is why the use of nano-emulsions is a suitable way to prevent side effects and increase the cytotoxicity of treatments.

Analyzing previous research, it has been observed that studies have been performed on the effect of citrus compounds and their nano-emulsions on cell lines (Shoorvarzi, Shahraki, Shafaei, Karimi, & Oskoueian, 2020). Pomelo leaves were chosen by *Liu et al.*, as suppliers of chlorophyll and carotenoids, to use their nano-emulsions as inhibitory agents for melanoma cells 375 and fibroblast cells CCD-986SK. Chlorophyll and carotenoids are natural pigments that own antioxidant, anti-inflammatory, and anticancer properties (Liu et al., 2021a). *Gasa-Falcon et al.*, studied the evolution of mandarin fiber incorporated in β-carotene nano-emulsions over gastrointestinal digestion (Gasa-Falcon, Odriozola-Serrano, Oms-Oliu, & Martin-Belloso, 2017).

Inhibition of tumor formation was studied by analyzing the effect of free and nano-encapsulated carotenoids and chlorophyll, respectively, on the pathways of cellular apoptosis (caspase-3, caspase-8, and caspase-9) and on proteins with a role in regulating cell cycle progression (cyclin A, cyclin B, cyclin-dependent kinases: CDK1 and CDK2, Bax,

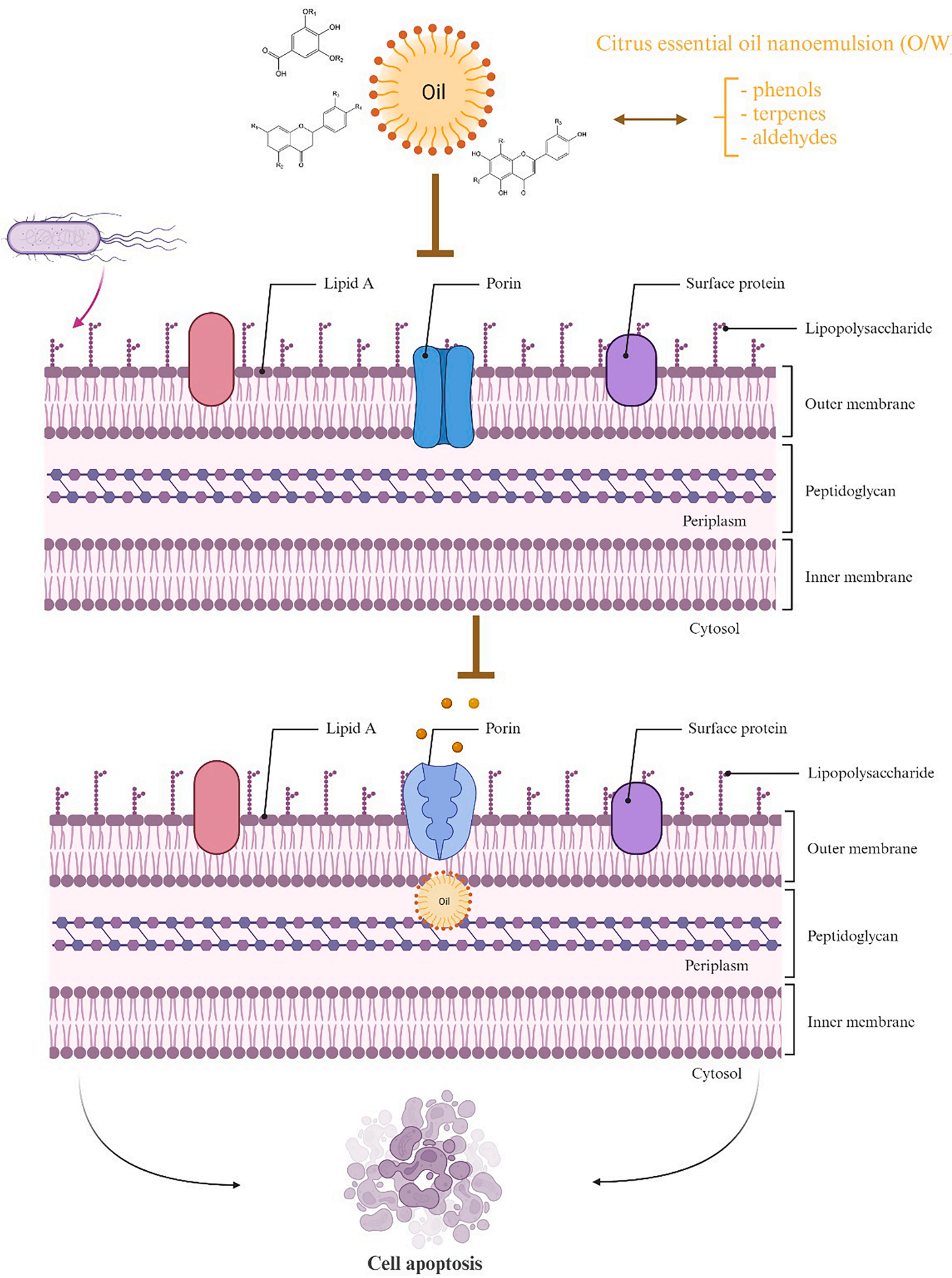

Fig. A4. Effect of citrus essential oils against gram-negative bacteria.

and p53). At 5, 10, and 20 g/mL doses, NEs produced metabolic changes in the components responsible for the integrity of melanoma cells and finally initiated the process of apoptosis and inhibiting tumor formation. In both cases, the growth of melanoma cells A375 stagnated at the G2/M cell phase checkpoint (Liu, Li, & Chen, 2021b) (Fig. A5).

The results of this research have demonstrated a higher potential for producing the apoptotic effect on melanoma cells A375. Additionally, the recovery of grapefruit fruit to obtain nanometric biological treatments brings a benefit to both, the medical and food industries (See Fig. A6).

Alkhatib et al., used in their research on orange essential oil nano-emulsions mixed with docetaxel (DOC-NEOO) to improve the antiproliferative effect of docetaxel (DOC), eliminate their side effects to increase their cardioprotective effect. The evaluation of these properties was performed following the application of nano-emulsions on mice (Alkhatib et al., 2017). To obtain relevant results, comparisons were made between control, individual and combined treatments. Briefly, the docetaxel load orange essential oil nano-emulsions have an anticancer effect due to the presence of orange oil which contains bioactive compounds with antioxidant activity and due to the nanosized particles which improve the permeation through membranes. Additionally, the ascorbic acid present in the orange oil composition, owns cardioprotective and cardiotoxicity reducing properties.

Bergamot essential oil and its nano-emulsions were used to evaluate

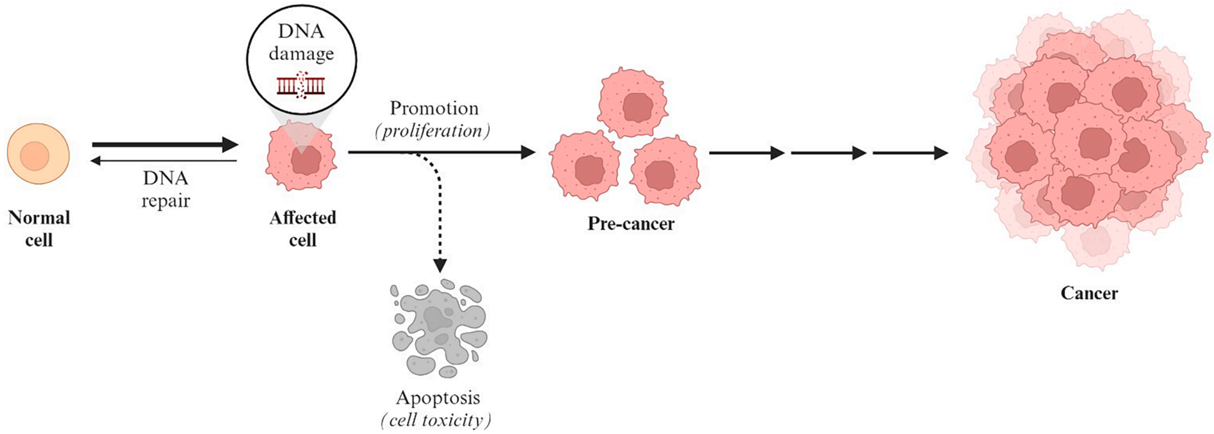

Fig. A5. Evolution of human cells in cancer disease.

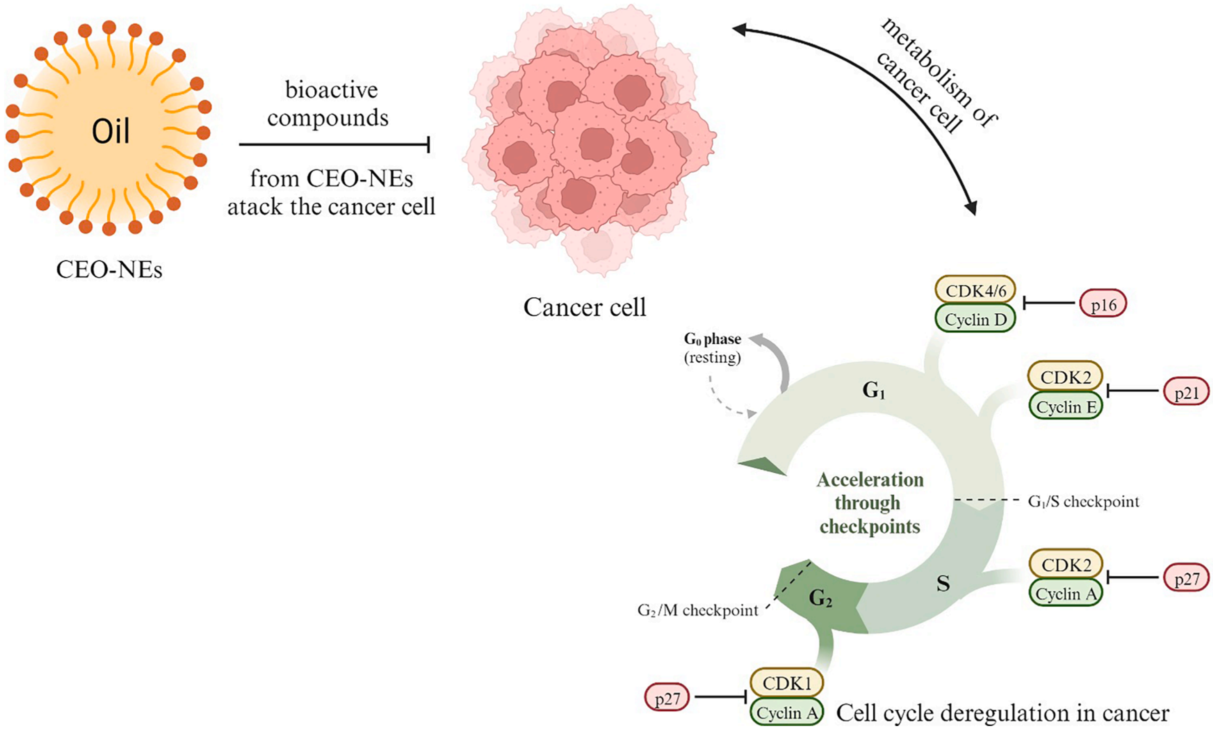

Fig. A6. Citrus essential oil nano-emulsions against cancer.

their cytotoxic effect against Caco-2 cells (epithelial cells isolated from colon tissue). The cells were treated with nano-emulsions containing 7,5, 15, 30, 75 mg/L pure essential oil. Using these formulas, was observed a reduction in the viability of Caco-2 cells, which suggests that bergamot oil nano-emulsions are suitable as anticancer agents (Marchese et al., 2020). *Sampaio et al.*, used lemon balm nano-emulsions against Caco-2 cells. The results showed that nano-emulsions decrease the viability of cancer cells (Sampaio et al., 2022). Lemon essential oil nano-emulsions were used with success as a programmed cellular death inducer in human lung cancer (Rad et al., 2020).

Antioxidant, antimicrobial and cytotoxic activity of citrus essential oils nano-emulsions are in a relationship of interdependence, determining each other. Essential oils contain radical scavengers and phytochemical antioxidants which increase antimicrobial and antitumor activity. Additionally, the nanometric size of particles allows the transport of bioactive compounds across membranes, facilitating targeted treatments against pathogenic microorganisms, yeasts, molds and cancer cells.

7. Conclusions

The physical and antimicrobial properties of CEO-NEs are dependent on the citrus species, properties and composition. Nano-emulsions can be successfully used in food matrices (fish, meat, dairy, fruits, vegetables and beverages) as flavoring agents, natural antimicrobial and antioxidant agents, and natural additives to increase shelf life. The goal of our work was to summarize out the properties of citrus essential oils nano-emulsions. In conclusion, encapsulated EOs in nano-emulsions offer several benefits in terms of physicochemical stabilization, biological activity, and product behavior, meeting consumer demand for healthy natural products. This review paper summed up some of the most recent topics concerning the biological properties of citrus essential oils nano-emulsions and citrus compounds nano-emulsions as well as their current and future applications. Based on the studied literature, it can be concluded that citrus essential oils-based nano-emulsions present interest for the food and pharmaceutic industry as functional ingredients, that can be exploited as natural source of bioactive compounds.

According to the data that was analyzed, it can be said that there is a scarcity of studies aimed at elucidating the antimutagenic effect of CEO-NEs, thus additional genes and cell lines must be further examined.

CRediT authorship contribution statement

Mădălina Lorena Medeleanu: Conceptualization, Investigation, Data curation, Methodology, Formal analysis, Software, Resources, Validation, Visualization, Writing – original draft. **Anca Corina Fărcaș:** Conceptualization, Methodology, Formal analysis, Software, Resources. **Cristina Coman:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Loredana Leopold:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Zorița Diaconeasa:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Sonia Ancuța Socaci:** Conceptualization, Methodology, Formal analysis, Software, Resources.

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.

Data availability

No data was used for the research described in the article.

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