Waste and Biomass Valorization
https://doi.org/10.1007/s12649-026-03520-3

ORIGINAL PAPER

Biovalorization of Citrus Peel Waste into Nano-Enhanced Natural Preservatives for Food Protection

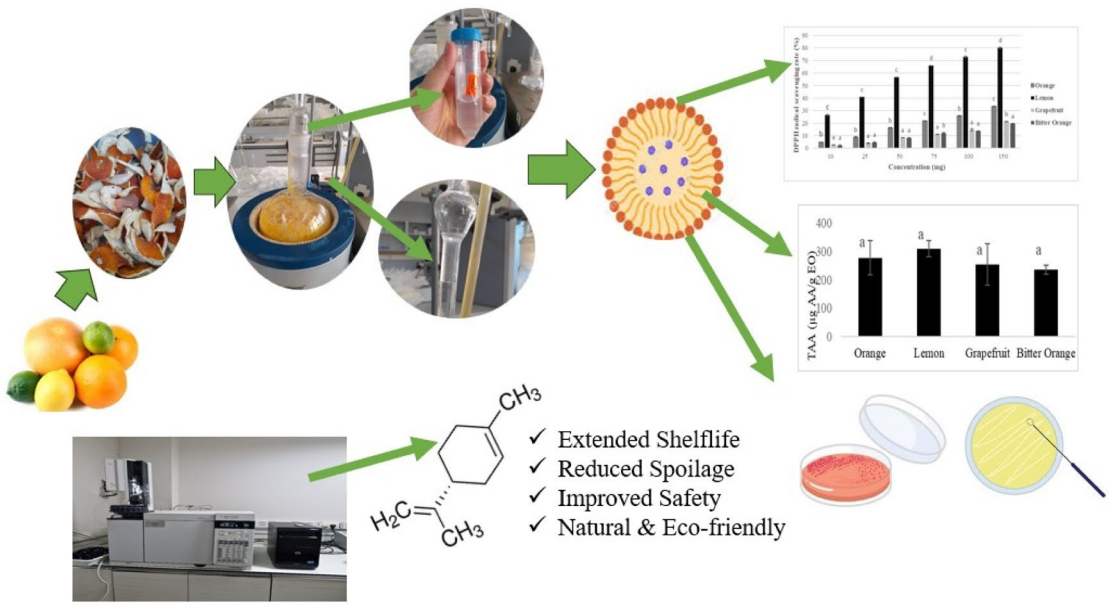
Yesim Ozogul[1] · Esmeray Kuley[1] · Gülsün Ozyurt[1] · Mustafa Durmuş[1] · Yılmaz Ucar[2] · Martina Čagalj[3] · Fethiye Takadaş[4] · Yetkin Sakarya[1] · Mediha Gürel[5,6] · Serya Tülin Özkütük[1] · Vida Šimat[3] · Fatih Ozogul[1,6]

Received: 4 August 2025 / Accepted: 2 February 2026

Abstract

Citrus essential oils (EOs) are well-known for their antimicrobial and antioxidant activities, but their direct use in foods is limited by volatility, instability, and poor water solubility. In this study, nanoemulsions (NEs) were developed using citrus peel waste as an eco-friendly emulsifier to improve EO stability and functionality. The major component in citrus EOs was D-limonene, ranging from 57.65% in lemon to 85.11% in bitter orange. The prepared NEs exhibited droplet sizes of 96.81–203.8 nm with low polydispersity (0.450–0.702), indicating uniform and stable dispersions. Nanoencapsulation significantly enhanced antioxidant activity, with total antioxidant values of 668–690 µg AAE/g NE compared to 235–309 µg AAE/g in free EOs. While free EOs showed stronger antimicrobial activity due to higher concentrations of active compounds, nanoemulsified EOs demonstrated notable bacteriostatic effects that increased with higher oil content. Lemon EO inhibited bacterial growth at 25 mg/mL, producing zone 32 mm, whereas its NE form yielded smaller zones of 8.5 and 13 mm against *Salmonella Paratyphi A* and *Pseudomonas putida*, respectively. Overall, citrus peel-based nanoemulsions provide a sustainable, effective delivery system for natural bioactives, enhancing antioxidant protection and microbial stability in foods, while also offering a promising strategy for valorizing agro-industrial citrus waste.Graphical abstract is preferred for publication in this journal. Please provide the graphical abstract.Please find the attachment for graphical abstract.

Graphical Abstract



Extended author information available on the last page of the article

Published online: 19 February 2026

Keywords Biovalorization · Citrus essential oil · Nanoemulsion · Antioxidant activity · Antimicrobial activity

Introduction

Citrus fruits are widely cultured and economically significant fruit crops globally. According to data from the United States Department of Agriculture (USDA), global citrus production reached approximately 101 million tonnes in the 2022/2023 marketing year. This comprised 48 million tonnes of oranges, 37 million tonnes of mandarins, 9 million tonnes of lemons, and 7 million tonnes of grapefruits [1]. Türkiye is an important citrus producer, yielding approximately 4.7 million tonnes annually, predominantly from the Aegean and Mediterranean regions. Particulary, the main part of the citrus cultivation and production is concentrated at the southern part of the country. In 2022, production included 1.9 million tonnes of mandarins, 1.3 million tonnes of lemons, 1.3 million tonnes of oranges, 198,000 tonnes of grapefruits, and 2,808 tonnes of bitter oranges [2].

The citrus processing industry generates large volumes of by-products, primarily peels, pulps, and seeds. Citrus peels, in particular, are easily accessible raw material that represent a rich source of macro- and micronutrients, including water, proteins, carbohydrates, minerals, essential oils, fibers, carotenoids, vitamin C, and phenolic compounds. These constituents give citrus peels significant bioactive properites, including antioxidant, antibacterial, antifungal, and anti-inflammatory properties [3–6]. As a result, citrus peels are increasingly valued matrices for reuse and valorization, contributing to waste reduction and the development of high-value industrial applications.

Essential oils (EOs), extracted from various plant tissues including citrus peels, involve volatile, aromatic and lipophilic compounds. These natural products, also known as volatile oils, essences, aetherolea, or etheric oils, are typically obtained via steam or hydro-distillation [7]. Plants produce EOs as part of their defense mechanisms against microbial pathogens such as bacteria, fungi, and viruses [8]. EOs have a long-standing history of use in perfumery, traditional medicine, antiseptics, and food preservation [9–12].

In recent years, citrus-derived EOs have gained attention as eco-friendly, cost-effective, and natural alternatives to synthetic preservatives like nitrates, benzoates, and sodium nitrites. Notably, the extraction of EOs from citrus peels, typically discarded as agro-industrial waste, offers a dual benefit: reducing environmental pollution and providing natural solutions for food preservation. These oils demonstrate broad-spectrum biological activity, including antimicrobial efficacy against both Gram-positive and Gram-negative bacteria [13–20].

However, the practical application of EOs in the food industry is limited by inherent challenges such as poor solubility in water, low bioavailability, rapid volatility, limited permeability, and inadequate long-term stability. To address these limitations, nanoencapsulation techniques, particularly oil-in-water (O/W) nanoemulsions (NE), have emerged as a promising technique for enhancing the stability, bioavailability, and functional performance of lipophilic bioactive compounds, such as Eos [21, 22]. These systems are thermodynamically unstable colloidal systems consisting of oil droplets dispersed in an aqueous phase, stabilized by surfactants or emulsifiers [23] used to enhance the physical stability, controlled release, and biological efficacy of encapsulated compounds [24–27]. Advances in high-energy methods (e.g., ultrasonication, high-pressure homogenization) and low-energy techniques (e.g., phase inversion, spontaneous emulsification) have enabled the production of nanoemulsions with droplet sizes typically below 200 nm and narrow polydispersity indices [28, 29]. Recent studies have focused on optimizing surfactant types, oil-to-water ratios, the use of natural emulsifiers and processing parameters to improve physical stability, minimize coalescence, enhance shelf life and biocompatibility [30–32]. The nanoemulsification of the EOs improves their antioxidant, antimicrobial, and anti-inflammatory properties compared to their free forms, thereby making them suitable for diverse applications in food conservation, cosmetics and pharmaceuticals [33–35]. The success uses of EOs and their O/W nanoemulsions in these industries depends on encapsulation efficiency, scaling up of the manufacturing processes at higher tecnological level, and their functional performances in complex matrices such as food systems and biological environments [36, 37].

Therefore, the objective of the present study was to extract EOs from lemon, grapefruit, orange, and bitter orange peels and characterize their volatile composition. To fully assess their potential as natural food preservatives, their antioxidant capacities were investigated. Furthermore, the antimicrobial activities of both the free and nanoencapsulated forms of these EOs were evaluated against foodborne pathogens and spoilage bacteria.

Material and Methods

Citrus Materials

Citrus fruits were harvested during the natural growing season (December-March) in Kozan/Adana/Turkey. Lemon (*Citrus limon* 'Enter'), grapefruit (*Citrus paradisi* 'Riolet'), orange (*Citrus sinensis* 'Washington Navel') and bitter orange (*Citrus aurantium* L.) were used. They were washed

thoroughly to remove any dirt. After that, a knife was used carefully to remove the outer layer of citrus fruits. The peels were sun dried.

Essential Oils

The dry citrus peels of lemon, grapefruit, orange and bitter orange were hydrodistilled in a Clavenger apparatus for 4 h. The isplated EOs were stored at −18 °C until the analyses.

GC–MS Analyses of Volatile Compounds of Circus Peels

The Agilent Brand 7890B GC, 7010B MS equipment was used to determine the volatile component analysis. A DB-Wax capillary column (60 m × 0.25 mm i.dx 0.25 µm, J&W Scientific-Folsom, USA) was filled with 1 µL of the DCM-diluted samples for the analysis. After four minutes at 40 °C, the temperature of the column was raised by 3 °C per minute to 90 °C, then by 4 °C per minute to 130 °C, and finally, after four minutes by 5 °C per minute to 240 °C, where it stayed for eight minutes. The temperature of the injection was 250 °C. Helium was the carrier gas used. The mass range was 30–600 m/z, while the electron energy was 70 eV. The split was 1:20. NIST14 database was used as the library (standard reference data).

Nanoemulsion Preparation

Nanoemulsions of citrus peel EOs were prepared using a high-energy ultrasonication technique. The method was adapted from the procedure described by Joe et al. [38], with minor modifications, based on the original protocol developed by Hamouda et al. [39]. The oil phase of the oil-in-water (O/W) nanoemulsion consisted of essential oil (4%, v/v), Tween 80 (3%, v/v) as a non-ionic surfactant, and ethanol (3%, v/v) as a co-solvent (all reagents were obtained from Sigma-Aldrich, Lyon, France). This mixture was incubated at 86 °C for 1 h to ensure complete homogenization. Subsequently, the oil phase was emulsified with distilled water to reach a final concentration of 90% (v/v) aqueous phase.

The resulting pre-emulsion was subjected to ultrasonic homogenization using a probe-type ultrasonic processor (Sonics, Newtown, CT, USA) operating at 500 W power and a frequency of 20 kHz. Sonication was carried out for 15 min at 90% amplitude. To prevent overheating and maintain emulsion stability, the beaker containing the mixture was surrounded by an ice bath, maintaining a constant temperature of approximately 15 °C. A total volume of 200 mL of nanoemulsion was prepared for each batch.

Physicochemical Analysis of the Nanoemulsions

The METU Central Laboratory at the Middle East Technical University in Ankara, Turkey, examined the physicochemical characteristics of nanoemulsions. The dynamic light scattering method (Zetasizer Nano ZS90, Malvern Instruments, Malvern, UK) was employed to determine the droplet size and polydispersity index (PDI) of the nanoemulsions. To prevent additional scattering impacts, nanoemulsions were diluted ten times with pure water before testing. A goniometer (Attension Theta, Biolin Scientific, Espoo, Finland) was used to measure surface tension.

Antioxidant Activity Assay of EOs and NEs

Total Antioxidant Activity

In vitro total antioxidant activity (TAA) of citrus essential oils (EOs) and their nanoemulsions (NE) was measured by the phosphomolybdenum method according to Prieto et al. [40]. TAA was defined as µg of ascorbic acid equivalents (AAE) per g of EO and NE.

DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of citrus essential oils and their nanoemulsions was assessed using the method described by Wu et al. [41], with slight modifications.

A solution of DPPH (0.1 mM) was prepared in 95% ethanol. For the assay, 1.5 mL of the DPPH solution was mixed with various concentrations of the test samples. The mixtures were incubated in the dark at room temperature for 60 min to allow the reaction to occur. Following incubation, the absorbance of the resulting solutions was measured at 517 nm using a UV–Vis spectrophotometer.

A decrease in absorbance indicated an increase in free radical scavenging activity. The percentage of DPPH radical scavenging was calculated using the following formula:

$$\text{DPPH Scavenging Activity}(\%) = \frac{A\ \text{control} - A\ \text{sample}}{A\ \text{control}} \times 100$$

where *A control* is the absorbance of the control (DPPH solution without sample), and *A sample* is the absorbance in the presence of the test sample. The IC50 value, which is the concentration of antioxidant required to scavenge 50% DPPH, was calculated from the inhibition curve.

Total Phenolic Content (TPC)

The TPC of EOs and NEs were measured by using the Folin-Ciocalteu method [42]. Results were reported as mg gallic

acid equivalents (GAE)/g oil, and the gallic acid standard had a concentration range of 20 to 1,000 µL. To achieve maximum solubilization, 20 µL of each EO was dissolved in DMSO (dimethylsulfoxide). The samples were then left at room temperature for complete reaction with the Folin–Ciocalteu reagent. After two hours in the dark absorbance of the sample was measured using a Shimadzu UV 1800 spectrophotometer (Kyoto, Japan) at 760 nm.

Antimicrobial Activity of Extracts

Bacterial Strains

Food-borne pathogenic Gram-positive (*Staphylococcus aureus* ATCC 33591, *Enterococcus faecalis*) and Gram-negative (Salmonella Paratyphi A NCTC13, *Camplylobacter jejuni* ATCC 33560, and *Pseudomonas aeruginosa*) bacteria were used provided by the National Collection of Type Cultures in London, United Kingdom, and the American Type Culture Collection in Rockville, Maryland, USA.

Agar Well Diffusion Method

Hwanhlem et al. [43] used the well diffusion technique to investigate the in vitro bactericidal ability of EO and NE forms on Mueller–Hinton Agar (MHA, Darmstadt, Germany). The cell density was arranged to 0.5 McFarland (10^8 cfu/ml) after the fish spoilage bacteria were grown for 24 h at 37 °C in nutrient broth. One hundred µL of bacterial cell culture was poured to a petri dish with Muller Hilton agar. Solid media was used to create four 5 mm wells. As a control, the wells were filled with distilled water and Muller Hinton Broth (MHB, Merck, Germany). The other wells received an inoculum of 50 µl of nanoemulsions. Callipers were used to quantify the zones of inhibition in millimeters following a 24-h incubation period at 37 °C in the petri dishes.

Minimum Inhibitory (MIC) and Bactericidal Concentration (MBC)

The microdilution technique developed by the Clinical and Laboratory Standards Institute (CLSI) was utilized to ascertain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). After being cultured for 24 h at 37 °C, the test microorganisms were standardized to a McFarland cell density of 0.5. MHB, Oxoid, CM0405 Mueller–Hinton Broth was used as the medium. Sterile tubes were used to dilute a stock solution of 100 mg/mL made from NE samples to 0.19 µg/mL. A tube containing MHB and bacterial suspension without samples was tested as a positive control, while a tube without MHB was analysed as a negative control. The other tubes containing MHB included test bacteria and diluted stock solutions. Following a number of preparations, the test tubes were incubated at 37 °C for 24 h. The test tubes' bacterial growth was contrasted with that of the control tubes. The tubes with no bacterial growth were inoculated into the MHA surface based on the MIC findings. In order to record the MBC values, the petri dishes were then incubated for 24 h at 37 °C.

Statistical Analysis

The statistical analysis was done by Duncan's Multiple Comparison Test (SPSS 22, Chicago, IL, USA) and one-way analysis of variance (ANOVA). A $P < 0.05$ value indicated a significant difference between the groups.

Results and Discussion

The Composition of Essential Oils of Citrus Peels

It is well-documented that the chemical properties of EOs is affected by a range of factors, including the plant's developmental stage, environmental conditions, genetic variability, and extraction methods [44–46]. In the present study, the volatile constituents of citrus peel essential oils were identified using gas chromatography–mass spectrometry (GC–MS), and the results are shown in Table 1.

Twenty-seven volatile compounds were identified in lemon peel EO, with D-limonene being the predominant component (57.65%), followed by γ-terpinene (13.38%), β-pinene (5.28%), p-cymene (4.52%), β-myrcene (4.37%), and α-pinene (2.88%). These results confirm the characteristic dominance of D-limonene in lemon EO, consistent with previous reports. For instance, Yeddes et al. [47] and Benoudjit et al. [48] reported D-limonene contents of 71.81% and 65%, respectively, in lemon peel oils. Similarly, Yazgan et al. [16] identified D-limonene (52.85%), p-cymene (14.36%), and β-pinene (13.69%) as major constituents. Akarca and Sevik [50] also observed D-limonene (68.65%) and γ-terpinene (10.8%) as dominant components. The abundance of D-limonene and other monoterpenes, which possess known antibacterial and antioxidant properties, underpins the widespread use of lemon EO as a food additive [51].

Grapefruit EO exhibited a high D-limonene content (77.65%), along with β-myrcene (5.90%), sabinene (2.94%), α-pinene (1.82%), and β-phellandrene (1.12%). These findings align with those of Ozogul et al. [18] who reported D-limonene as the major compound (82.86%) in grapefruit EO. Similarly, Tran et al. [52] found D-limonene (71.21%) and γ-terpinene (17.68%) as principal constituents. Miya et al. [53] analyzed grapefruit peels from three South

Table 1 The volatile compounds of essential oils of citrus peels

Chemical compounds (%)	Lemon	Grapefruit	Orange	Bitter orange	RT	LRI
α-pinene	2.88	1.82	1.83	1.79	11.168	1120
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- (synonym: alpha-thujene)	1.36				11.352	1025
β- pinene	5.28			0.78	14.947	1127
Sabinene	1.04	2.94	1.77	0.40	15.573	1142
3-Carene	0.30		0.78		17.001	1177
β-myrcene	4.37	5.90	6.19	6.27	17.698	1194
4-Carene	0.58				18.526	1214
D-Limonene	57.65	77.64	83.49	85.11	19.889	1247
β-Phellandrene	0.77	1.12	1.01	1.01	20.129	1253
Eucalyptol	0.27				20.233	1255
γ-Terpinene	13.38		1.12		21.912	1296
1,3,6-Octatriene, 3,7-dimethyl-, (Z)- (synonym: cis-β-Ocimene)		0.89		0.80	22.039	1299
p-Cymene	4.52				22.836	1318
Cyclohexene, 1-methyl-4-(1-methylethylidene)- (synonym: Terpinolene)	1.35				23.594	1337
Octanal		2.68	0.95	0.81	23.635	1338
Nonanal		0.36			28.310	1451
Benzene, 1-methyl-4-(1-methylethenyl)- (synonym: p-Cymenene)	0.75				29.917	1470
2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, cis- (synonym: (Z)-Linalool oxide (furan))		0.69			30.204	1477
6-Octenal, 3,7-dimethyl-, (R)-	0.34	0.57			31.677	1514
Decanal		1.39	0.72	0.60	32.681	1539
Copaene		0.39			33.240	1549
Linalool	0.55	0.74	1.30	1.53	34.415	1576
Linalyl acetate				0.38	35.122	1593
Tricyclo[2.2.1.0(2,6)]heptane-3-methanol, 2,3-dimethyl- (synonym:Teresantalol)	0.20				35.416	1600
Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	0.75	0.60			36.712	1630
Terpinen-4-ol					37.206	1642
Caryophyllene	0.34	0.86			37.814	1656
2,6-Octadienal, 3,7-dimethyl-, (Z)- (synonym:β-Citral)	0.19				40.205	1713
α-Terpineol	0.52	0.55		0.52	40.779	1732
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)- (synonym: Geranyl acetate)	0.42				41.835	1762
5,9-Undecadien-1-yne, 6,10-dimethyl- (synonym: cis-.beta.-Farnesene)	0.45	0.39			41.985	1766
β-Bisabolene (synonym: (+)-Valencene/β-Bisabolene)	0.58				42.400	1778
4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate (synonym: (−)-Lavandulyl acetate)	0.25				42.851	1791
δ-Cadinene		0.47			43.505	1809
Benzeneacetamide,.alpha.-hydroxy-N,.alpha.-dimethyl-	0.18				51.507	2037

RT retention time, *LRI* linear retention index

African cultivars and reported D-limonene levels ranging from 87 to 90%. The predominance of D-limonene is significant due to its demonstrated anti-inflammatory, antioxidant, antimicrobial, and potential anticancer properties [54, 55].

The major components identified in orange EO were D-limonene (83.49%), β-myrcene (6.19%), α-pinene (1.83%), sabinene (1.77%), linalool (1.30%), γ-terpinene (1.12%), and β-phellandrene (1.01%). This compositional profile is comparable to that reported by Akarca and Sevik [50], who found limonene (95.51%) to be the primary constituent, followed by β-myrcene (1.98%) and α-pinene (0.76%). Duman et al. [56] similarly characterized Turkish Thomson orange peel EO and identified limonene (71.80%) and β-myrcene (4.55%) as the main constituents. Farahmandfar et al. [57] analyzed essential oil composition across different drying methods for Thomson navel oranges and observed limonene (71.54%), β-myrcene (7.20%), and linalool (4.11%) as dominant compounds, concluding that freeze-drying best preserved EO composition.

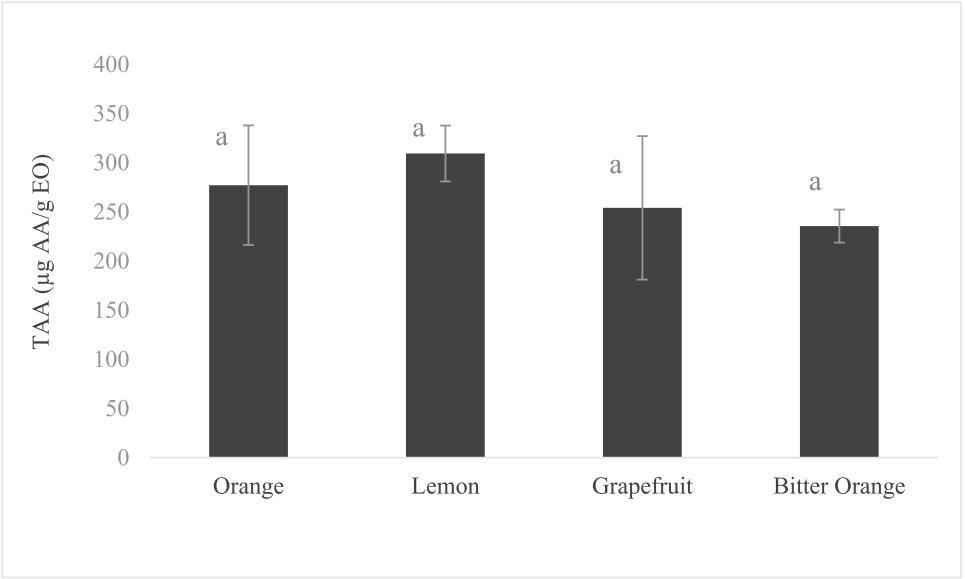


Fig. 1 Total antioxidant contents of citrus essential oils (µg AA/g EO)

Bitter orange EO contained D-limonene (85.11%), β-myrcene (6.27%), α-pinene (1.79%), and linalool (1.53%). This is consistent with the findings of Farahmandfar et al. [57], who reported limonene (85.49%), linalool (2.74%), and β-myrcene (0.97%) in bitter orange EO. Similarly, Heydari et al. [58] found limonene (60%), linalool (11%), and myrcene (6%) in EO derived from bitter orange peel waste. Bitter orange EO is commonly utilized in the fragrance, pharmaceutical, and food industries due to its aromatic and bioactive properties, including notable antibacterial and antioxidant effects [59, 60].

While the primary chemical constituents identified in this study—particularly D-limonene, β-myrcene, and α-pinene—are consistent with previous reports, the relative proportions varied. Such differences may be attributed to both intrinsic and extrinsic factors. Intrinsic variables include species, genetic background, and physiological maturity, while extrinsic factors encompass environmental conditions (e.g., climate, soil type), geographic origin, and extraction methodology [60–62]. These variations highlight the importance of standardized protocols and source traceability in EO research and industrial applications.

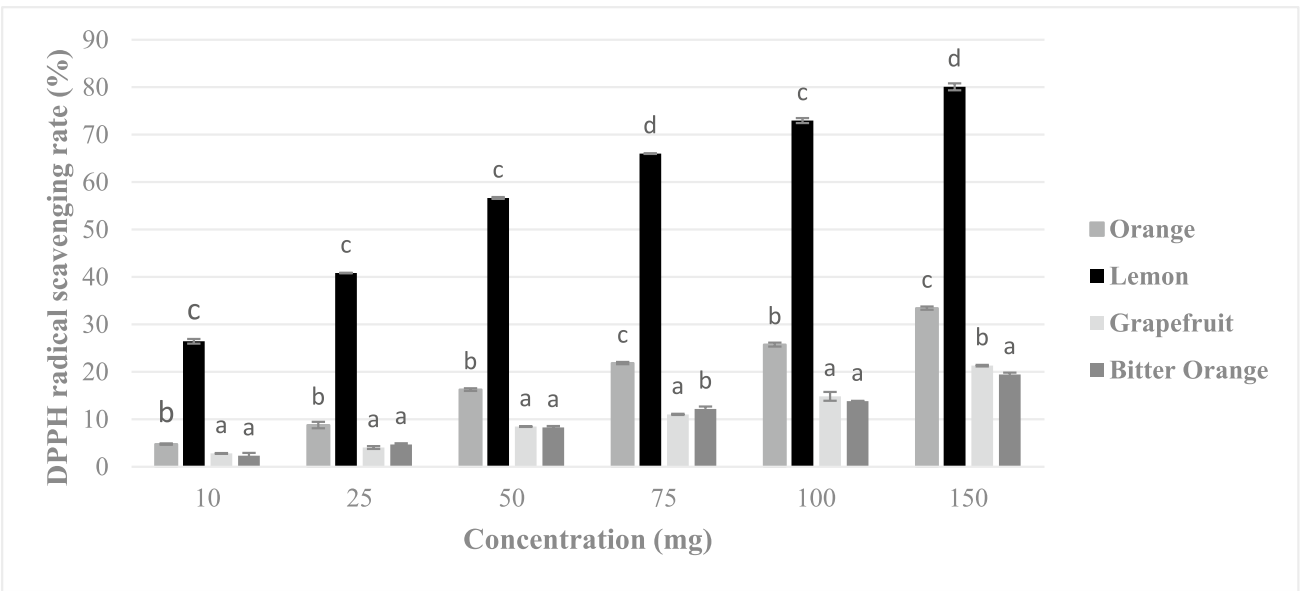


Fig. 2 DPPH scavenging capacity of citrus peel essential oils (%)

Antioxidant Activity of Citrus EOs

The total antioxidant activity (TAA) of EOs extracted from citrus peels is shown in Fig. 1. Among the tested samples, lemon peel EO exhibited the highest TAA with a value of 309.42 µg ascorbic acid equivalents per gram (mg AA/g); however, the differences among all tested groups were not statistically significant ($P > 0.05$). These findings are partially supported by Güzel and Akpınar [63]. who reported higher ascorbic acid content in lemon peel compared to orange, mandarin, and other citrus species. Similarly, Asinruth et al. [64] reported 218 µg AAE/g and 276 µg AAE/g TAA in essential oils obtained from *Citrus limon* (lemon) and *Citrus limetta* (mosambi) peels, respectively.

Figure 2 shows the DPPH radical scavenging activity of citrus peel EOs, expressed as IC_{50} values. The IC_{50} values, representing the concentration required to inhibit 50% of the DPPH radicals, were determined to be 49.01 µg/mL for lemon, 222.08 µg/mL for orange, 363.24 µg/mL for grapefruit, and 397.21 µg/mL for bitter orange EOs. Lower IC_{50} values indicate greater antioxidant efficacy; thus, lemon EO demonstrated the highest radical scavenging activity. These results align with those of El Aboubi et al. [65], who reported IC_{50} values for lemon peel EO ranging from 40.57 to 100.22 µg/mL. In contrast, Çoban [66] reported higher IC_{50} values of 20.13 mg/mL for lemon and 37.66 mg/mL for orange peels, suggesting differences possibly due to plant origin or extraction conditions.

A dose-dependent increase in DPPH radical scavenging activity was observed for all citrus peel EOs. Lemon EO consistently exhibited the highest inhibitory effect across all tested concentrations. Similar results were presented by Li et al. [67], who indicated an increase in DPPH inhibition from 20 to 80% for lemon EO in the concentration range of 16–144 mg/mL, and up to 30% for sweet orange EO. Haraoui et al. [68] also reported that citrus extracts showed high anti-radical activity, with inhibition values ranging from 57.58 to 95.41% for fruit juices and 50.21 to 84.69% for leaf extracts.

Overall, the data obtained in this study confirm that citrus peel essential oils contain bioactive compounds capable of effectively donating protons to neutralize free radicals, demonstrating substantial antioxidant capacity. Lemon EO, in particular, showed the most potent antioxidant activity, making it a good candidate for applications in food preservation and functional product development.

The total phenolic content analysis can be used as an important indicator of antioxidant potential, which can be used as an initial screening for essential oils when used as natural sources of antioxidants in foods [33]. Lemon essential oil (20.94 mg GAE/g) had the greatest TPC in this study, followed by grapefruit essential oil (15.32 mg GAE/g), bitter orange essential oil (15.19 mg GAE/g), and orange essential oil (12.19 mg GAE/g). It was shown that grapefruit EO exhibited the highest total phenolic content (77.3 mg GAE/g), followed by orange EO (49.8 mg GAE/g) and lemon EO (35.6 mg GAE/g) [69]. Similarly, Grapefruit EO had the greatest TPC (44.32 mg GAE/g), followed by lemon EO (35.52 mg GAE/g), bitter orange EO (32.44 mg GAE/g), and orange EO (31.99 mg GAE/g), according to Durmuş et al. [70]. Abeysinghe et al. [71] found that the TPC of *C. unshiu* (Satsuma mandarins), *C. sinensis* (orange) and *C. reticulata* (mandarin), varied from 18.5 to 38.5%. Additionally, they revealed that the major phenolic component was hesperidin.

Antimicrobial Activity of Citrus EOs

Inhibition Zone of EO Against Test Bacteria

The antimicrobial activity of citrus peel essential oils was evaluated using the agar well diffusion method, and the inhibition zone diameters are presented in Table 2. Statistically significant differences were observed among the inhibition

Table 2 Inhibition zone of citrus essential oils against test bacteria (mm)

	Orange	Lemon	Grapefruit	Bitter orange
Food-borne bacteria				
Methicillin-resistant *Staphylococcus aureus*	–	$41.00 \pm 3.16^{*a}$	38.25 ± 2.36^{a}	6.50 ± 0.63^{b}
Salmonella Paratyphi A	–	36.50 ± 1.73^{a}	29.75 ± 1.71^{b}	10.25 ± 0.96^{c}
Klebsiella pneumoniae	–	38.50 ± 3.51^{a}	30.00 ± 1.41^{b}	6.75 ± 0.50^{c}
Enterococcus faecalis	–	31.00 ± 3.16^{a}	33.50 ± 2.38^{a}	9.75 ± 0.50^{b}
Fish spoilage bacteria				
Vibrio vulnificus	–	32.25 ± 1.71^{a}	30.75 ± 2.99^{a}	21.50 ± 3.00^{b}
Pseudomonas putida	–	39.00 ± 1.83^{a}	33.50 ± 1.29^{b}	22.25 ± 1.50^{c}
Photobacterium damselae	–	45.25 ± 3.40^{a}	32.50 ± 2.65^{b}	12.75 ± 0.96^{c}

*Mean (n = 3) ± Standard deviation. [a–c]Display significant differences ($P < 0.05$) between groupS in a column.—No inhibition zones

zones of the essential oils tested ($P < 0.05$). Lemon and grapefruit EOs demonstrated the most potent antibacterial effects, while orange EO showed negligible activity against all tested bacterial strains, exhibiting no measurable inhibition zones.

Lemon EO exhibited inhibition zones ranging from 31 mm against *E. faecalis* to 41 mm against *S. aureus*. Similarly, grapefruit EO produced inhibition zones between 29 mm (Salmonella Paratyphi A) and 38 mm (*S. aureus*). These findings align with Saleem and Saeed (2020) also observed superior antibacterial activity of lemon peel extract compared to orange, particularly against Gram-negative bacteria. *Klebsiella pneumoniae*, for instance, showed 28 mm inhibition zones in response to lemon EO.

In contrast, although Dubey et al. [72] reported antibacterial activity of orange peel EO against various pathogens such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *E. coli*, and *S. aureus*, no such activity was observed in the present study. The discrepancy could be due to differences in extraction methods, peel maturity, or cultivar origin. Supporting the current findings, Han et al. [73] demonstrated broad-spectrum antimicrobial activity of grape peel EO extract against both bacterial and fungal strains.

Bitter orange EO exhibited limited antibacterial effect, with the maximum inhibition zone of 10 mm observed against *Salmonella* Paratyphi A. The inhibitory effect of bitter orange extract against fish spoilage bacteria was higher than that against food-pathogens. *Photobacterium damselae* and *Pseudomonas putida* were the most sensitive bacterium against lemon extract with an inhibition zone of 45.25 and 39.00 mm, respectively. Lemon and grapefruit EOs showed statistically comparable antimicrobial effects against *Vibrio vulnificus*. In contrast, bitter orange EO exhibited the weakest activity against *P. damselae* (12.75 mm), with inhibition zones exceeding 20 mm for other spoilage bacteria.

Previously, Shehata et al. [74] demonstrated that sweet orange peel extract had the strongest antimicrobial activity against every microorganism tested, with the exception of *Aspergillus carbonarious* ITEM 5010. Lemon peel showed less antimicrobial activity against the same pathogens than sweet orange peel. With the exception of *Yersinia enterocolitica* ATCC 23715, grapefruit peel extract shown remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria.

Minimum Inhibitory (MIC) and Bactericidal Concentration (MBC)

MIC and MBC values of citrus peel EOs against foodborne pathogens and spoilage bacteria are shown in Table 3. Lemon EO exhibited the lowest MIC values, followed by grapefruit EO, indicating strong bacteriostatic effects. In contrast, orange EO showed minimal antimicrobial efficacy, with MIC and MBC values exceeding 100 mg/mL across all tested strains. Lemon EO inhibited MRSA (methicillin-resistant *Staphylococcus aureus*) and *Salmonella* Paratyphi A at a concentration of 25 mg/mL, while the MIC values for *K. pneumoniae* and *E. faecalis* were 50 mg/mL. Previously, Saleem and Saeed [75] reported a MIC value of 130 mg/mL for lemon extract against *Klebsiella pneumoniae*, indicating that antibacterial activity may vary depending on the extract's composition, particularly the concentrations

Table 3 Minimum Inhibitory (MIC) and Bactericidal Concentration (MBC) (mg/mL)

	Orange		Lemon		Grapefruit		Bitter orange	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Food-borne pathogens								
Methicillin-resistant *Staphylococcus aureus*	> 100	> 100	25	100	50	100	100	> 100
Salmonella Paratyphi A	> 100	> 100	25	100	50	> 100	100	> 100
Klebsiella pneumoniae	> 100	> 100	50	> 100	50	> 100	100	> 100
Enterococcus faecalis	> 100	> 100	50	100	50	100	100	> 100
Fish spoilage bacteria								
Vibrio vulnificus	> 100	> 100	25	100	50	100	100	> 100
Pseudomonas putida	> 100	> 100	25	> 100	50	100	100	> 100
Photobacterium damselae	> 100	> 100	25	100	50	> 100	100	> 100

Table 4 Properties of oil-in-water nanoemulsions based on essential oils

	Orange	Lemon	Grapefruit	Bitter Orange
Mean droplet size (nm)	96.81	203.8	149.3	187.9
Polydispersity index	0.512	0.450	0.702	0.695
Surface tension (N/m)	42.46 ± 0.06	41.08 ± 0.18	42.76 ± 0.33	41.73 ± 0.19

of zinc, magnesium, and phenolic compounds. According to Ozogul et al. [18], grape fruit peel EO demonstrated a strong bacteriostatic impact against *Salmonella* Parathypi A and *Pseudomonas luteola*, whereas it had a moderate effect against *Vibrio vulnificus*, *Seratia liquefaciens, S. aureus*, and *E. faecalis*.

Regarding spoilage bacteria, lemon EO showed MIC values of 25 mg/mL against *V. vulnificus*, *P. putida*, and *P. damselae*. Grapefruit and bitter orange EOs exhibited MIC values of 50 mg/mL and 100 mg/mL, respectively, against all tested bacteria. The orange and bitter orange extracts' bactericidal dosages exceeded 100 mg/mL. These results are consistent with findings by Ozogul et al. [18], who observed bacteriostatic effects of grapefruit EO against *S. Paratyphi A* and *Pseudomonas luteola*. According to Uysal et al. [76], grape fruit peel oil has also been shown to inhibit *S. typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 13315, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, and *Serratia marcescens* ATCC 8100, with the exception of *P. aeruginosa* ATCC 27853. Kamel et al. [77] found that orange fruit peel extract (petroleum ether and methanol extract) had strong activity against Gram-positive *S. aureus*, but little activity against Gram-negative *E. coli* and *P. aeruginosa*, and no activity against *Candida albicans*. In the current study, apart from *K. pneumonia* and *P. putida,* lemon EO showed bactericidal effect against all bacteria at dose of 100 mg/mL. Similarly, the MBC value of grapefruit extract was found as 100 mg/mL, with the exception of *S.* Paratyphi A, *K. pneumonia*, and *P. damselae*.

Encapsulation of Citrus Essential Oils by Nanoemulsion

Physicochemical Analysis of the Nanoemulsions

The physicochemical properties of nanoemulsions formulated with citrus peel essential oils are presented in Table 4**,** including droplet size, polydispersity index (PDI), and surface tension.

The droplet size of the nanoemulsions ranged from 96.81 nm (orange EO) to 203.08 nm (lemon EO). These sizes fall within the typical nanoemulsion range of 20–200 nm [78], although slightly higher values observed may be attributed to differences in oil type, surfactant concentration, and emulsification technique. Durmus [79] reported smaller droplet sizes (47.40–94.66 nm) for nanoemulsions prepared from citrus EOs, suggesting that high-pressure homogenization may yield finer emulsions. Similarly, Ozogul et al. [80] reported droplet diameters of 63.02 nm for rosemary EO and 112.82 nm for thyme EO.

PDI values ranged from 0.450 to 0.702, indicating moderately polydisperse systems. Higher PDI values reflect a broader distribution of droplet sizes, which may reduce the long-term stability of nanoemulsions. In contrast, Charnvanich et al. [29] observed PDI values near 0.1 for emulsions prepared using high-pressure homogenization, indicating more uniform droplet size distribution. Factors such as emulsification technique, surfactant-to-oil ratio, and homogenization speed significantly affect droplet uniformity [28, 30].

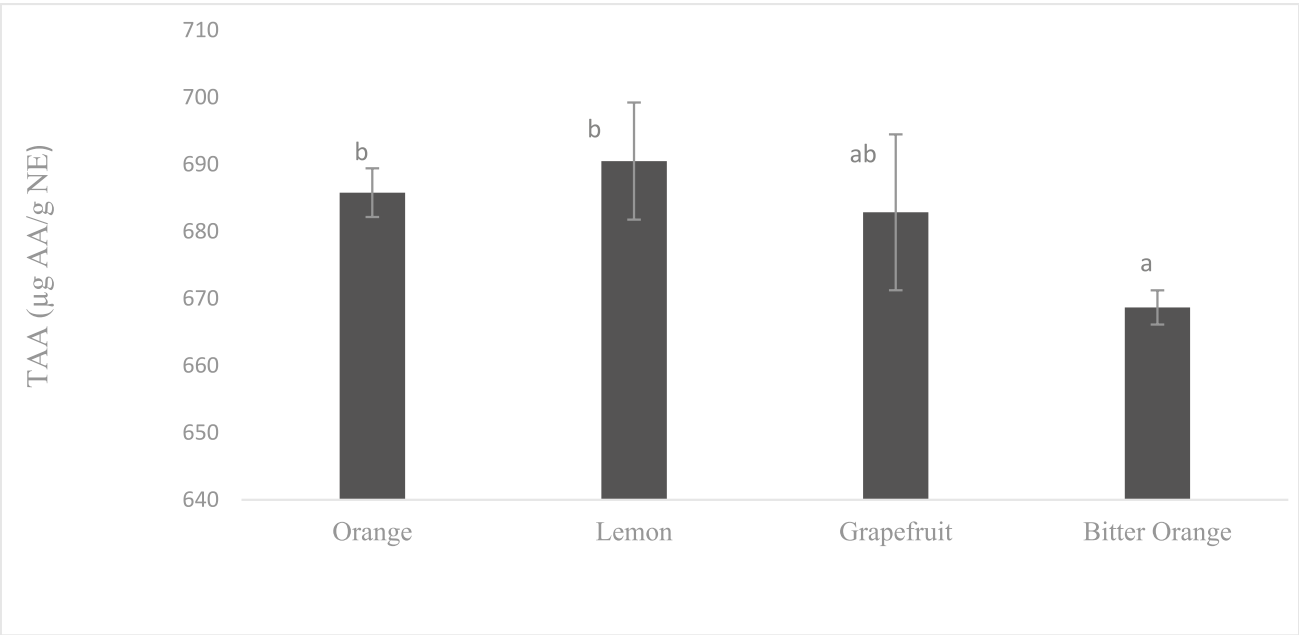


Fig. 3 Total antioxidant activity of citrus essential oil nanoemulsions (µg AA/g nanoemulsion)

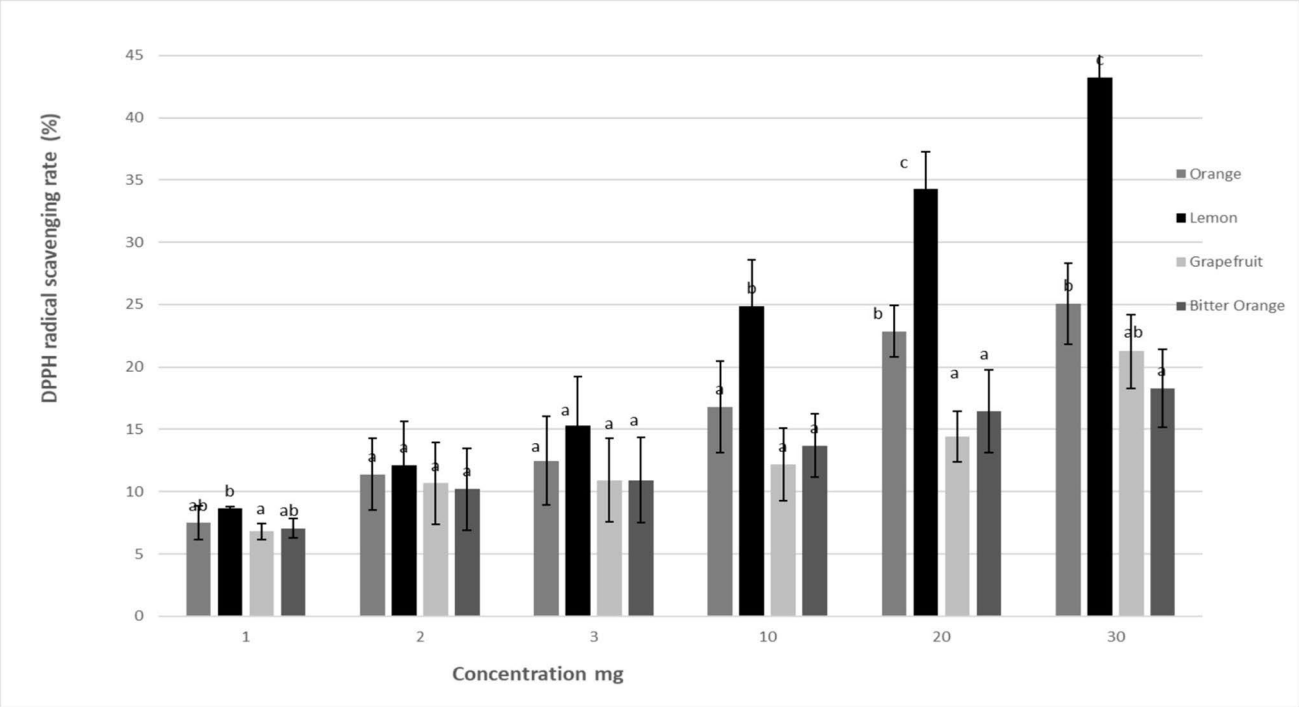


Fig. 4 DPPH radical scavenging capacity of citrus essential oil nanoemulsions (% inhibition). Data are presented as mean ± SD (n = 3). Different letters (a–c) indicate statistically significant differences ($P < 0.05$)

Surface tension values varied among the samples, with the lowest value recorded for lemon EO nanoemulsion (41.08 N/m) and the highest for grapefruit EO nanoemulsion (42.76 N/m). These values are higher than those reported by Durmus [79], who found surface tension values between 35.15 and 39.18 N/m for citrus-based nanoemulsions. Differences in surface tension are largely influenced by the chemical composition of the oils, surfactant properties, and processing parameters [32, 81].

Antioxidant Activity of Citrus Essential Oil Nanoemulsions

The antioxidant potential of the nanoemulsified forms of citrus essential oils (EOs) was evaluated using total antioxidant activity (TAA), DPPH radical scavenging capacity, and total phenolic content (TPC) assays. These analyses aimed to assess whether nanoencapsulation could enhance the bioactivity of citrus EOs.

Figure 3 shows the TAA values of citrus EO nanoemulsions. Among the samples, the lemon EO nanoemulsion exhibited the highest antioxidant activity, and no statistically significant difference was observed between orange and grapefruit nanoemulsions ($P > 0.05$). Bitter orange EO nanoemulsion had the lowest TAA value. Also, nanoemulsified citrus EOs displayed at least double the TAA compared to their non-encapsulated forms. These findings suggest that nanoencapsulation significantly improves the antioxidant potential of citrus EOs, likely by enhancing their solubility and dispersion in aqueous environments.

The DPPH radical scavenging capacity of the nanoemulsified EOs is shown in Fig. 4. A concentration-dependent increase in scavenging activity was observed for all tested nanoemulsions (orange, lemon, grapefruit, and bitter orange), mirroring the trend seen in their free EO counterparts. The IC_{50} values of the nanoemulsions were determined as 34.37 µg/mL for lemon, 62.11 µg/mL for orange, 104.43 µg/mL for grapefruit, and 136.60 µg/mL for bitter orange. Lemon EO nanoemulsion demonstrated the strongest radical scavenging activity, as indicated by its lowest IC_{50} value, consistent with results obtained from the free EO forms.

Overall, the nanoemulsion forms exhibited lower IC_{50} values and therefore higher antioxidant capacities compared to their free forms. These results are in agreement with prior studies. Liu et al. [33] reported that nanoencapsulation significantly enhanced the DPPH scavenging ability of lemon EO. Similarly, Alam et al. [82] demonstrated greater antioxidant activity in nanoemulsified cinnamon EO than in its free form. The enhanced antioxidant effect observed in nanoemulsions is often attributed to increased surface area and improved dispersion of EO droplets, which facilitates more efficient interaction with free radicals [83].

Phenolic compounds are widely recognized for their antioxidant properties. In this study, the TPC values of citrus

Table 5 Inhibition zone of nanoemulsions (NE) against test bacteria (mm)

	Orange NE	Lemon NE	Grapefruit NE	Bitter orange NE
Food-borne bacteria				
Methicillin-resistant *Staphylococcus aureus*	–	–	–	–
Salmonella Paratyphi A	–	8.50 ± 0.71^{a}	6.75 ± 0.35^{b}	–
Klebsiella pneumoniae	–	–	10.50 ± 0.71^{a}	7.50 ± 0.71^{b}
Enterococcus faecalis	–	–	–	–
Fish spoilage bacteria				
Vibrio vulnificus	11.50 ± 0.58^{a}	–	–	8.33 ± 0.58^{b}
Pseudomonas putida	–	13.00 ± 0.71^{a}	7.75 ± 0.35^{b}	–
Photobacterium damselae	8.75 ± 0.50^{a}	–	8.50 ± 0.41^{a}	8.13 ± 0.63^{ab}

*Mean (n = 3) ± Standard deviation. [a–c]Display significant differences ($P < 0.05$) between groups in a column.—No inhibition zones

EO nanoemulsions were determined as follows: 2.54 mg GAE/g for lemon, 2.31 mg GAE/g for orange, 2.05 mg GAE/g for grapefruit, and 3.03 mg GAE/g for bitter orange. These results are substantially lower than the TPC values reported for free EOs in other studies. For example, Sir Elkhatim et al. [69] reported TPC levels in citrus peels as 77.3 mg GAE/g for grapefruit, 49.8 mg GAE/g for orange, and 35.6 mg GAE/g for lemon. Durmus et al. [70] similarly observed higher TPC in free EO forms, 44.32, 35.52, 32.44, and 31.99 mg GAE/g for orange, bitter orange, lemon, and grapefruit, respectively. The reduced phenolic content in the nanoemulsion formulations may be attributed to the lower quantity of essential oil incorporated during the emulsification process. Comparable findings were reported by Sabry et al. [84], who measured TPC values of 2.89 mg GAE/g and 0.74 mg GAE/g in nanoemulsions derived from orange and tangerine oils, respectively.

Antimicrobial Activity of Nanoemulsions

The antimicrobial activity of citrus EO nanoemulsions against foodborne pathogens and fish spoilage bacteria is shown in Table 5. The effectiveness of nanoemulsions varied according to both the type of citrus oil and the bacterial strain. There were no inhibition zones observed for *S. aureus* (MRSA) or *E. faecalis*, indicating resistance to these formulations. These findings are consistent with Kara et al. [85], who reported no antibacterial activity of hemp seed oil and aloe vera nanoemulsions against tested bacterial strains like *Listeria monocytogenes, E. coli, Staphylococcus aureus, Salmonella enterica* and *Vibrio parahaemolyticus*. However, contrasting results have been reported elsewhere. Wang et al. [36] found that grapefruit seed extract and its nanoemulsified form significantly inhibited biofilm formation by *Escherichia coli* and *S. aureus*. Similarly, Kang et al. [86] observed that citrus EO nanoemulsions exhibited high antimicrobial activity against *S. aureus*, *E. coli*, *Saccharomyces cerevisiae* and *Bacillus subtilis*.

In the present study, *Salmonella Paratyphi A* was resistant to orange and bitter orange nanoemulsions but exhibited modest inhibition zones of 8.5 mm and 6.75 mm when treated with lemon and grapefruit nanoemulsions, respectively. *Klebsiella pneumoniae* showed sensitivity only to grapefruit and bitter orange nanoemulsions, with inhibition zones of 10.50 mm and 7.50 mm, respectively.

These results differ from some literature findings that report greater susceptibility of Gram-positive bacteria to EO nanoemulsions compared to Gram-negative strains [82]. The enhanced sensitivity of Gram-positive bacteria is generally attributed to the disruption of their phospholipid-rich membrane by EO constituents. In Gram-negative bacteria, hydrophilic nanoemulsion droplets may pass through porin channels in the outer membrane, but are less likely to cause membrane destabilization [37]. Encapsulation of D-limonene in an organogel-based nanoemulsion has been reported to enhance antibacterial activity by damaging cytoplasmic membranes, resulting in structural degradation of microbial cells [87].

Among the spoilage bacteria tested, lemon EO nanoemulsion exhibited a measurable zone of inhibition (13 mm) only against *P. putida*. Yazgan et al. [16] previously reported inhibition zones ranging from 14.75–19.25 mm for fish spoilage bacteria and 14.38–24.25 mm for foodborne pathogens when treated with lemon EO nanoemulsions. In our study, orange and bitter orange nanoemulsions showed inhibition zones of 11.50 mm and 8.33 mm, respectively, against *V. vulnificus*. *Photobacterium damselae* showed resistance to lemon nanoemulsion but exhibited moderate sensitivity to other formulations, with inhibition zones exceeding 8 mm.

Durmus [79] found that mandarin and grapefruit nanoemulsions were more effective than orange and lemon in reducing bacterial loads in rainbow trout fillets. Consistent with this, our results showed that grapefruit EO

Table 6 Minimum Inhibitory (MIC) and Bactericidal Concentration (MBC) of nanoemulsions (NE) (mg/mL)

	Orange		Lemon		Grapefruit		Bitter orange	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Food-borne pathogens								
Methicillin-resistant *Staphylococcus aureus*	100	> 100	100	> 100	100	> 100	25	> 100
Salmonella Paratyphi A	50	100	25	100	25	100	100	100
Klebsiella pneumoniae	100	> 100	100	> 100	100	> 100	100	> 100
Enterococcus faecalis	100	> 100	100	> 100	100	> 100	100	> 100
Fish spoilage bacteria								
Vibrio vulnificus	100	> 100	100	> 100	100	> 100	100	> 100
Pseudomonas putida	50	> 100	100	> 100	100	> 100	100	> 100
Photobacterium damselae	100	> 100	100	> 100	100	> 100	100	> 100

nanoemulsion formed inhibition zones of 7.75 mm and 8.50 mm against *P. putida* and *P. damselae*, respectively.

Overall, the antimicrobial efficacy of nanoemulsified citrus EOs was lower than that of their pure forms. This finding is consistent with earlier studies [18, 49], although other reports suggest that nanoencapsulation can enhance EO antimicrobial activity [35, 36, 88]. In the present study, nanoemulsions contained only 4% EO, which may explain their reduced effectiveness, as lower concentrations of active antimicrobial compounds were present.

The MIC and MBC values of citrus EO nanoemulsions against various bacterial strains are presented in Table 6. Orange nanoemulsion showed MIC values of 50 mg/mL against *S. Paratyphi* A and *P. putida*, and 100 mg/mL for all other tested strains. The lowest MIC values (25 mg/mL) were recorded for lemon and grapefruit nanoemulsions against *S*. Paratyphi A.

Listeria innocua and *S. aureus* exhibited the greatest sensitivity, being inhibited at 25 mg/mL, whereas *S. typhi, E. coli*, and *P. aeruginosa* required higher concentrations [89]. Although grapefruit EO nanoemulsion did not exhibit bactericidal effects, it did show bacteriostatic activity against *S. Paratyphi A*, *P. luteola*, *Serratia liquefaciens*, and *P. damselae*, as also observed by Ozogul et al. [18]. Yazgan et al. [16] found that both nanoemulsified and pure lemon EO at concentrations ≥ 25 mg/mL showed considerable bactericidal activity against spoilage and pathogenic bacteria. In the present study, bitter orange nanoemulsion generally demonstrated bacteriostatic activity at 100 mg/mL, except for MRSA, which showed susceptibility at 25 mg/mL. The bactericidal concentration for most strains was > 100 mg/mL, with the exception of *S.* Paratyphi A, where an MBC of 100 mg/mL was recorded.

These results further support the notion that while nanoencapsulation offers formulation advantages, the reduction in EO concentration may limit its antimicrobial potency unless optimized for higher loading or targeted delivery.

Conclusions

This study demonstrated that nanoemulsification is an effective approach to enhance the stability and functional properties of citrus essential oils (EOs) derived from citrus peel waste. The predominant compound identified in citrus EOs was D-limonene, ranging from 57.65% in lemon to 85.11% in bitter orange. The prepared nanoemulsions exhibited favorable physicochemical characteristics, including droplet sizes of 96.81–203.8 nm and low polydispersity indices (0.450–0.702), confirming their stability and uniformity. Nanoencapsulation significantly improved the antioxidant potential of citrus EOs, with total antioxidant activity values (668–690 µg AAE/g NE) nearly twice those of their free counterparts (235–309 µg AAE/g EO), indicating enhanced bioavailability and radical scavenging efficiency. Lemon EO exhibited strong bacteriostatic activity, with a minimum inhibitory concentration of 25 mg/mL and inhibition zones up to 32 mm against all tested bacterial strains. In contrast, its nanoemulsion form produced smaller inhibition zones of 8.5 mm and 13 mm against Salmonella Paratyphi A and *Pseudomonas putida*, respectively. Although free EOs displayed stronger antimicrobial effects, likely due to higher concentrations of active components, nanoemulsified EOs maintained notable bacteriostatic activity that increased proportionally with oil content.

Overall, citrus peel-based nanoemulsions present a cost-effective, environmentally sustainable, and efficient delivery system for natural bioactives, aligning with circular bioeconomy principles. These formulations have strong potential as natural preservatives to extend the shelf life and microbial stability of perishable foods. Future studies should aim to optimize formulation parameters, increase EO loading capacity, and assess performance in real food matrices to support their broader industrial application.

Author Contributions YO, FO, and VŠ contributed to the study conception and design. EK, GO, MD, YU, MČ, FT, YS, MG, and STÖ performed material preparation, data collection, analysis and writing original draft. YO and GO, and VŠ performed supervision, editing and review the manuscript. All authors read and approved the final manuscript.

Funding This research was financed by the Scientific and Technological Research Council of Turkey (TUBITAK); Grant No: 123N064 (PRIMA Programme Sect. "Material and Methods"). This project was supported by the PRIMA program under project InnoSol4Med (Project ID 1836). The PRIMA programme is supported by the European Union.

Data Availability Data will be made available on request.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication All authors consent to the publication of the manuscript.

Competing interest The authors have no competing interest to declare.

References

1. USDA: The United States (https.//apps.fas.usda.gov/psdonline/app/index). (2023).
2. TUIK: Turkish Statistical Institute, Product report, Citrus fruits. Published 26 May 2023. Access link: https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-1.Tahmini-2023-49534. (2023).
3. Alsakhawy, S.A., Baghdadi, H.H., El-Shenawy, M.A., El-Hosseiny, L.S.: Comparative phytochemical composition and antimicrobial activity of citrus peel essential oils and phenolic compounds. Anti Infect. Agents **21**(4), 57–68 (2023)
4. Habeeb, H., & Thoppil, J. E.: Vitamin C and Citrus peels—a treasure chest for healthy life. In Herbal Formulations, Phytochemistry and Pharmacognosy (pp. 401–411). Elsevier (2024).
5. Seyyedi-Mansour, S., Carpena, M., Donn, P., Barciela, P., Perez-Vazquez, A., Echave, J., Prieto, M.A.: Citrus seed waste and circular bioeconomy: insights on nutritional profile, health benefits, and application as food ingredient. Appl. Sci. **14**(20), 9463 (2024)
6. Hu, Y., Wang, F., Chen, H., Chen, L., Liu, Y.: Integrated nutritional and functional components analyses reveal insights into the peel and pulp quality at different harvest times of 'Dahongpao'tangerine (*Citrus reticulata* Blanco). Food Chem. **463**, 141263 (2025)
7. Pheko-Ofitlhile, T., Makhzoum, A.: Impact of hydrodistillation and steam distillation on the yield and chemical composition of essential oils and their comparison with modern isolation techniques. J. Essen. Oil Res. **5**, 1–11 (2024)
8. de Sousa, D.P., Damasceno, R.O.S., Amorati, R., Elshabrawy, H.A., de Castro, R.D., Bezerra, D.P., Lima, T.C.: Essential oils: chemistry and pharmacological activities. Biomolecules **13**(7), 1144 (2023)
9. Gurkok, S., Sezen, S.: Uses of essential oils in different sectors. Essential Oils Extraction Methods Appl. **5**, 207–228 (2023)
10. Agnihotry, S., Chopra, D., Singh, J., Negi, S., Srivastav, A. K., Upadhyay, J., & Sharma, G.: Aromatherapy evolution and blending basics of essential oils. In Aromatherapy: The Science of Essential Oils (pp. 1–30). Bentham Science Publishers (2024).
11. Misra, P., Pandey, G., Pandey, S., Singh, A., Chaurasia, A. K., Lal, E. P., Shukla, P. K.: Plant as potential resources for efficacious essential oils: Underpinning aromatherapy evolution. In Aromatherapy: The Science of Essential Oils (pp. 31–63). Bentham Science Publishers (2024).
12. Udourioh, G.A., Bazza, B.M., Pilani, M.P., Solomon, M.M.: Therapeutic characteristics of essential oils: historical and scientific considerations. J. Appl. Sci. Environ. Manage. **29**(2), 569–579 (2025)
13. Hou, T., Sana, S.S., Li, H., Xing, Y., Nanda, A., Netala, V.R., Zhang, Z.: Essential oils and its antibacterial, antifungal and antioxidant activity applications: a review. Food Biosci. **47**, 101716 (2022)
14. Jackson-Davis, A., White, S., Kassama, L.S., Coleman, S., Shaw, A., Mendonca, A., London, L.: A review of regulatory standards and advances in essential oils as antimicrobials in foods. J. Food Prot. **86**(2), 100025 (2023)
15. Ersanli, C., Tzora, A., Skoufos, I., Fotou, K., Maloupa, E., Grigoriadou, K., Zeugolis, D.I.: The assessment of antimicrobial and anti-biofilm activity of essential oils against *Staphylococcus aureus* strains. Antibiotics. **12**(2), 384 (2023)
16. Yazgan, H., Ozogul, Y., Kuley, E.: Antimicrobial influence of nanoemulsified lemon essential oil and pure lemon essential oil on food-borne pathogens and fish spoilage bacteria. Int. J. Food Microbiol. **306**, 108266 (2019)
17. Raspo, M.A., Vignola, M.B., Andreatta, A.E., Juliani, H.R.: Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the USA. Food Biosci. **36**, 100651 (2020)
18. Ozogul, Y., Ozogul, F., Kulawik, P.: The antimicrobial effect of grapefruit peels essential oil and its nanoemulsion on fish spoilage bacteria and food-borne pathogens. LWT **136**, 110362 (2021)
19. Al-Deen, R.B., Aloklah, B., Al-Amir, L.: Chemical composition and antibacterial activity of the peel essential oils extracted from citrus fruits. J. Agri. Appl. Bio **2**(2), 114–123 (2021)
20. Dhouibi, I., Flamini, G., Bouaziz, M.: Comparative study on the essential oils extracted from Tunisian rosemary and myrtle: chemical profiles, quality, and antimicrobial activities. ACS Omega **8**(7), 6431–6438 (2023)
21. McClements, D.J.: Advances in nanoparticle and microparticle delivery systems for increasing the dispersibility, stability, and bioactivity of phytochemicals. Biotechnol. Adv. **38**, 107287 (2020)
22. Jaiswal, K.K., Kumar, V., Vlaskin, M.S., Nanda, M., Verma, M., Ahmad, W., Kim, H.: Hydropyrolysis of freshwater macroalgal bloom for bio-oil and biochar production: kinetics and isotherm for removal of multiple heavy metals. Environ. Technol. Innov. **22**, 101440 (2021)
23. Silva, H.D., Cerqueira, M.A., Vicente, A.A.: Influence of surfactant and processing conditions in the stability of oil-in-water nanoemulsions. J. Food Eng. **167**, 89–98 (2015)
24. Ferreira, C.D., Nunes, I.L.: Oil nanoencapsulation: development, application, and incorporation into the food market. Nanoscale Res. Lett. **14**, 1–13 (2019)
25. Sharma, R., Nath, P.C., Das, P., Rustagi, S., Sharma, M., Sridhar, N., Sridhar, K.: Essential oil-nanoemulsion based edible coating: Innovative sustainable preservation method for fresh/fresh-cut fruits and vegetables. Food Chem. **460**, 140545 (2024)
26. Hashem, A.H., Doghish, A.S., Ismail, A., Hassanin, M.M., Okla, M.K., Saleh, I.A., Shehabeldine, A.M.: A novel nanoemulsion based on clove and thyme essential oils: characterization, antibacterial, antibiofilm and anticancer activities. Electron. J. Biotech. **68**, 20–30 (2024)
27. Koch, P., Dhua, S., Mishra, P.: Critical review on Citrus essential oil extracted from processing waste-based nanoemulsion: preparation, characterization, and emerging food application. J. Essent. Oil Res. **36**(5), 407–425 (2024)

28. Jadhav, R.P., Koli, V.W., Kamble, A.B., Bhutkar, M.A.: A review on nanoemulsion. Asian J. Res. Pharma. Sci **10**(2), 103–108 (2020)
29. Charnvanich, D., Singpanna, K., Panapisal, V.: Formulation and optimization of nanoemulsions loaded with gamma−aminobutyric acid (GABA) for dermatological application: assessing skin permeation and penetration enhancement. Cosmetics **11**(1), 19 (2024)
30. Kundu, P., Arora, K., Gu, Y., Kumar, V., Mishra, I.M.: Formation and stability of water-in-oil nano-emulsions with mixed surfactant using in-situ combined condensation-dispersion method. The Can. J. Chem. Engin. **97**(7), 2039–2049 (2019)
31. Su, J., Yang, M., He, Z., Guan, Y., Fu, S.: The effect of particle size and dispersity index on the drying behavior of colloidal microdroplets. Colloids Surf. A Physicochem. Eng. Asp. **708**, 135965 (2025)
32. Musakhanian, J., Osborne, D.W.: Understanding microemulsions and nanoemulsions in (Trans) dermal delivery. AAPS PharmSciTech **26**(1), 1–36 (2025)
33. Liu, H., Qiu, N., Ding, H., Yao, R.: Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res. Int. **41**(4), 363–370 (2008)
34. Alam, A., Ansari, M.J., Alqarni, M.H., Salkini, M.A., Raish, M.: Antioxidant, antibacterial, and anticancer activity of ultrasonic nanoemulsion of *Cinnamomum cassia* L. essential oil. Plants **12**(4), 834 (2023)
35. Özogul, Y., El Abed, N., Özogul, F.: Antimicrobial effect of laurel essential oil nanoemulsion on food-borne pathogens and fish spoilage bacteria. Food Chem. **368**, 130831 (2022)
36. Wang, W., Leng, Z., Liu, Q., Zhao, J., Li, S.: Nano-emulsification of Osmanthus essential oil: characterizations, stability and molecular interactions explaining antibacterial activity. Ind. Crops Prod. **219**, 118987 (2024)
37. Medeleanu, M.L., Fărcaş, A.C., Coman, C., Leopold, L., Diaconeasa, Z., Socaci, S.A.: Citrus essential oils–based nano-emulsions: functional properties and potential applications. Food Chem. X **20**, 100960 (2023)
38. Joe, M.M., Chauhan, P.S., Bradeeba, K., Shagol, C., Sivakumaar, P.K., Sa, T.: Influence of sunflower oil based nanoemulsion (AUSN-4) on the shelf life and quality of Indo-Pacific king mackerel (*Scomberomorus guttatus*) steaks stored at 20°C. Food Control **23**(2), 564–570 (2012)
39. Hamouda, T., Hayes, M.M., Cao, Z., Tonda, R., Johnson, K., Wright, D.C., Baker, J.R.: A novel surfactant nanoemulsion with broad-spectrum sporicidal activity against Bacillus species. J. Infec. Diseases. **180**(6), 1939–1949 (1999)
40. Prieto, P., Pineda, M., Aguilar, M.: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. **269**(2), 337–341 (1999)
41. Wu, H.C., Chen, H.M., Shiau, C.Y.: Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. **36**(9), 949–957 (2003)
42. Gamez-Meza, N., Noriega-Rodriguez, J.A., Medina-Juarez, L.A., Ortega-Garcia, J., Cazarez-Casanova, R., Angulo-Guerrero, O.: Antioxidant activity in soybean oil of extracts from Thompson grape bagasse. J. Am. Oil Chem. Soc. **76**, 1445–1447 (1999)
43. Hwanhlem, N., Ivanova, T., Haertlé, T., Jaffrès, E., Dousset, X.: Inhibition of food-spoilage and foodborne pathogenic bacteria by a nisin Z-producing *Lactococcus lactis* subsp. *lactis* KT2W2L. LWT **82**, 170–175 (2017)
44. Farhadi, N., Babaei, K., Farsaraei, S., Moghaddam, M., Pirbalouti, A.G.: Changes in essential oil compositions, total phenol, flavonoids and antioxidant capacity of *Achillea millefolium* at different growth stages. Indust. Crops Pro **152**, 112570 (2020)
45. Mahajan, M., Kuiry, R., Pal, P.K.: Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants. J. Appl. Res. Med. Aromat. Plants **18**, 100255 (2020)
46. da Silva, W.M.F., Kringel, D.H., de Souza, E.J.D., da Rosa Zavareze, E., Dias, A.R.G.: Basil essential oil: methods of extraction, chemical composition, biological activities, and food applications. Food Bioprocess. Tech. **15**(1), 1–27 (2022)
47. Yeddes, W., Mejri, I., Grati Affes, T., Khammassi, S., Hammami, M., Aidi-Wannes, W., Saidani Tounsi, M.: Combined effect of essential oils from Clove (*Syzygium aromaticum* (L.) Merr. & LM Perry), Thyme (*Thymus vulgaris* L.) and Lemon peel (*Citrus limon* (L.) Osbeck) on anti-bacterial, cytotoxic and anti-inflammatory activities. Trends Phytochem. Res. **6**(1), 11–18 (2022)
48. Benoudjit, F., Hamoudi, I., Aboulouz, A.: Extraction and characterization of essential oil and hydrolate obtained from an Algerian lemongrass (*Cymbopogon citratus*). Algerian J. Environ. Sci. Tech. **8**(1), 63 (2022)
49. Yazgan, H.: Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. LWT **130**, 109669 (2020)
50. Akarca, G., Sevik, R.: Biological activities of *Citrus limon* L. and *Citrus sinensis* L. peel essential oils. J. Essent. Oil-Bearing Plants **24**(6), 1415–1427 (2021)
51. Dao, T.P., Tran, N.Q., Tran, T.T.: Assessing the kinetic model on extraction of essential oil and chemical composition from lemon peels (*Citrus aurantifolia*) by hydro-distillation process. Mater. Today Proc. **51**, 172–177 (2022)
52. Tran, T. H., Dao, T. P., Le, X. T., Huynh, B. L., & Minh, L. T. N.: Volatile compounds of grapefruit (*Citrus grandis* (L.) Osbeck) peel essential oil by cold pressing and hydrodistillation methods. In IOP Conference Series: Earth and Environmental Science, 1241 (1) p. 012070. IOP Publishing (2023).
53. Miya, G., Nyalambisa, M., Oyedeji, O., Gondwe, M., Oyedeji, A.: Chemical profiling, toxicity and anti-inflammatory activities of essential oils from three grapefruit cultivars from KwaZulu-Natal in South Africa. Molecules **26**(11), 3387 (2021)
54. Anandakumar, P., Kamaraj, S., Vanitha, M.K.: D-limonene: a multifunctional compound with potent therapeutic effects. J. Food Biochem. **45**(1), e13566 (2021)
55. Narayanankutty, A., Visakh, N.U., Sasidharan, A., Pathrose, B., Olatunji, O.J., Al-Ansari, A., Ramesh, V.: Chemical composition, antioxidant, anti-bacterial, and anti-cancer activities of essential oils extracted from *Citrus limetta* Risso peel waste remains after commercial use. Molecules **27**(23), 8329 (2022)
56. Duman, E., Soltanbeigi, A., Ozcan, M.M.: Chemical compositions of essential oil of some *Citrus* spp. (sour, lemon, kumquat, mandarin and orange) peels. J. Med. Spice Plants. **21**(4), 153–159 (2016)
57. Farahmandfar, R., Tirgarian, B., Dehghan, B., Nemati, A.: Changes in chemical composition and biological activity of essential oil from (*Citrus sinensis* L. Osbeck) peel under freezing, convective, vacuum, and microwave drying methods. Food Sci. Nutr. **8**(1), 124–138 (2020)
58. Heydari, M., Rostami, O., Mohammadi, R., Banavi, P., Farhoodi, M., Sarlak, Z., Rouhi, M.: Hydrodistillation ultrasound-assisted green extraction of essential oil from bitter orange peel wastes: optimization for quantitative, phenolic, and antioxidant properties. J. Food Process. Preserv. **45**(7), e15585 (2021)
59. Farag, M.A., Abib, B., Ayad, L., Khattab, A.R.: Sweet and bitter oranges: an updated comparative review of their bioactives, nutrition, food quality, therapeutic merits and biowaste valorization practices. Food Chem. **331**, 127306 (2020)
60. Gaff, M., Esteban-Decloux, M., Giampaoli, P.: Bitter orange peel essential oil: a review of the different factors and chemical

reactions influencing its composition. Flavour Fragr. J. **35**(3), 247–269 (2020)
61. Yingngam, B.: Chemistry of essential oils. In: Flavors and fragrances in food processing: Preparation and characterization methods (pp. 189–223). American Chemical Society (2022).
62. Khan, S., Sahar, A., Tariq, T., Sameen, A., & Tariq, F.: Essential oils in plants: Plant physiology, the chemical composition of the oil, and natural variation of the oils (chemotaxonomy and environmental effects, etc.). In Essential Oils (pp. 1–36). Academic Press (2023).
63. Güzel, M., Akpınar, Ö.: Turunçgil kabuklarının biyoaktif bileşenleri ve antioksidan aktivitelerinin belirlenmesi. Gümüşhane Üni. Fen Bilimleri Dergisi **7**(2), 153–167 (2017)
64. Asinruth, M., Renuka, R., Ragunathan, R., Johney, J.: Extracion of essential oil from *Citrus limon* and *Citrus limetta*: characterisation and antioxidant activity. World J. Pharma. Res. **14**(7), 1087–1103 (2025)
65. El Aboubi, M., Hdech, D.B., Bikri, S., Benayad, A., El Magri, A., Aboussaleh, Y.: Chemical composition of essential oils of *Citrus limon* peel from three Moroccan regions and their antioxidant, anti-inflammatory, antidiabetic and dermatoprotective properties. J. Herbmed Pharmacol. **12**(1), 118–127 (2022)
66. Çoban, E. Ç.: Antioxidant and antibacterial properties of orange and lemon peels (Master's thesis, Institute of Science and Technology) (2019).
67. Li, Y., Liu, S., Zhao, C., Zhang, Z., Nie, D., Tang, W., Li, Y.: The chemical composition and antibacterial and antioxidant activities of five citrus essential oils. Molecules **27**(20), 7044 (2022)
68. Haraoui, N., Allem, R., Chaouche, T.M., Belouazni, A.: In-vitro antioxidant and antimicrobial activities of some varieties citrus grown in Algeria. Adv. Tradit. Med. **20**(1), 23–34 (2020)
69. Sir Elkhatim, K.A., Elagib, R.A., Hassan, A.B.: Content of phenolic compounds and vitamin C and antioxidant activity in wasted parts of Sudanese citrus fruits. Food Sci. Nutr. **6**(5), 1214–1219 (2018)
70. Durmus, M., Özogul, Y., Ozyurt, G., Ucar, Y., Kosker, A.R., Yazgan, H., Özogul, F.: Effects of citrus essential oils on the oxidative stability of microencapsulated fish oil by spray-drying. Front. Nutr. **9**, 978130 (2023)
71. Abeysinghe, D.C., Li, X., Sun, C.D., Zhang, W.S., Zhou, C.H., Chen, K.S.: Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. Food Chem. **104**, 1338–1344 (2007)
72. Dubey, D., Balamurugan, K., Agrawal, R.C., Verma, R., Jain, R.: Evalution of antibacterial and antioxidant activity of methanolic and hydromethanolic extract of sweet orange peels. Recent Res. Sci. Tech. **3**(11), 63 (2011)
73. Han, C., Shao, H., Zhou, S., Mei, Y., Cheng, Z., Huang, L., Lv, G.: Chemical composition and phytotoxicity of essential oil from invasive plant, *Ambrosia artemisiifolia* L. Ecotoxicol. Environ. Saf. **211**, 111879 (2021)
74. Shehata, M.G., Awad, T.S., Asker, D., El Sohaimy, S.A., Abd El-Aziz, N.M., Youssef, M.M.: Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. Curr. Res. Food Sci. **4**, 326–335 (2021)
75. Saleem, M., Saeed, M.T.: Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent. J. King Saud Univ. Sci. **32**(1), 805–810 (2020)
76. Uysal, B., Sozmen, F., Aktas, O., Oksal, B.S., Kose, E.O.: Essential oil composition and antibacterial activity of the grapefruit (*Citrus paradisi*. L) peel essential oils obtained by solvent-free microwave extraction: comparison with hydrodistillation. Int. J. Food Sci. Technol. **46**(7), 1455–1461 (2011)
77. Kamel, F., Sabir, S., Mahal, A., Wei, X.: In vitro antibacterial activity of orange peel oil extract from *Citrus sinensis* fruit in Erbil. Egypt. J. Chem. **65**(4), 157–160 (2022)
78. Elsewedy, H.S.: Insights of nanoemulsion as a drug delivery system: an overview of current trends and applications. Indian J. Pharm. Educ. Res. **59**(2), 1–21 (2025)
79. Durmus, M.: The effects of nanoemulsions based on citrus essential oils (orange, mandarin, grapefruit, and lemon) on the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets at 4±2 C. J. Food Saf. **40**(1), e12718 (2020)
80. Ozogul, Y., Yuvka, I., Ucar, Y., Durmus, M., Kösker, A.R., Öz, M., Ozogul, F.: Evaluation of effects of nanoemulsion based onherb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) fillets during ice storage. LWT **75**, 677–684 (2017)
81. Ozogul, Y., Karsli, G.T., Yazgan, H., Kuley, E., Oztop, H.M., Ozogul, F., Esatbeyoglu, T.: Enhanced pathogen control through thymol and carvacrol nanoemulsions: a microfluidization approach. Food Bioprocess Tech. **6**, 1–11 (2025)
82. Allam, O.G., Ramadan, M.M., Ismail, S.A., Hebeish, A.: Preparation and application of lemon peel oil (*Citrus aurantifolia*) to improve microbial resistance of wool and viscose fabrics. Egypt. J. Chem. **65**(8), 511–521 (2022)
83. Noori, S., Zeynali, F., Almasi, H.: Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food Control **84**, 312–320 (2018)
84. Sabry, B.A., Badr, A.N., Mohammed, D.M., Desoukey, M.A., Farouk, A.: Validating the protective role of orange and tangerine peel extracts foramending food safety against microorganisms' contamination using molecular docking. Heliyon (2024). https://doi.org/10.1016/j.heliyon.2024.e27737
85. Kara, A., Caglak, E., Karsli, B.: Optimization and bioactive properties of nanoemulsion formulated with hemp (*Cannabis sativa* L.) seed oil and *Aloe vera* (*Barbadensis miller Stockton*) gel. ChemistrySelect **9**(17), e202305110 (2024)
86. Kang, Z., Chen, S., Zhou, Y., Ullah, S., Liang, H.: Rational construction of citrus essential oil nanoemulsion with robust stability and high antimicrobial activity based on combination of emulsifiers. Inno. Food Sci. Emerging Tech. **80**, 103110 (2022)
87. Zahi, M.R., Liang, H., Yuan, Q.: Improving the antimicrobial activity of D-limonene using a novel organogel-based nanoemulsion. Food Control **50**, 554–559 (2015)
88. Zheng, H., Chen, S., Liu, Z., Zhou, G., Zhang, X., Kang, S., Liu, Z.: Utilization of nanoemulsion to enhance antibacterial capacity of *Zanthoxylum bungeanum* pericarp essential oils against foodborne pathogenic bacteria. LWT **207**, 116657 (2024)
89. Zanganeh, H., Shahidi, F., Mortazavi, S.A., Alizadeh Behbahani, B.: Evaluation of antioxidant and antimicrobial properties of nanoemulsion of *Citrus paradisi* essential oil against pathogenic microorganisms: in vitro study. Iran. Food Sci. Technol. Res. J. **19**(4), 415–425 (2023)
90. Clinical and Laboratory Standards Institute, CLSI.: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Wayne, PA, USA (2008).

Authors and Affiliations

Yesim Ozogul[1] · Esmeray Kuley[1] · Gülsün Ozyurt[1] · Mustafa Durmuş[1] · Yılmaz Ucar[2] · Martina Čagalj[3] · Fethiye Takadaş[4] · Yetkin Sakarya[1] · Mediha Gürel[5,6] · Serya Tülin Özkütük[1] · Vida Šimat[3] · Fatih Ozogul[1,6]

✉ Yesim Ozogul
yozogul@cu.edu.tr

[1] Faculty of Fisheries, Department of Seafood Processing Technology, University of Cukurova, 011330 Balcalı, Adana, Turkey

[2] Department of Forestry, Vocational School of Aladag, Cukurova University, 01330 Aladag, Balcalı, Adana, Turkey

[3] University Department of Marine Studies, University of Split, R. Boškovića 37, 21000 Split, Croatia

[4] Vocational School, Department of Chemical Technology, University of Alanya, 07400 Antalya, Turkey

[5] Electronic and Automation Department, University of Bitlis Eren, 13000 Bitlis, Turkey

[6] Biotechnology Research and Application Center, University of Cukurova, 01330 Adana, Turkey