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Preparation, Characterization and Bioactive Evaluation of Citrus Peels Essential Oils Nanoemulsions

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Abstract

In this study, essential oils were extracted from orange and lemon peels were irradiated at doses of 0, 2, and 4 kGy, with 4 kGy demonstrating highest antioxidant and antimicrobial effects. Nano emulsions (NEs) were prepared using the sonication technique with Tween 80 and water. Characterization studies were showed particle sizes ranging from 29.92 to 47.12 nm for orange NE and 17.6 to 64.81 nm for lemon NE. TEM images confirmed that the nanoparticles were spherical, uniformly dispersed, and non-aggregated, with sizes aligning with DLS measurements. The antioxidant activity of CEO-NEs, assessed by DPPH%, increased with irradiation from 17.3 to 19.3% for orange and 15.5 to 21.9% for lemon NE. The FRAP values ranged from 764.5 to 840.7 µM TE/mg for orange and 1011.9 to 1138.9 µM TE/mg for lemon NE. Lemon NE exhibited stronger antioxidant and antimicrobial activities than orange, demonstrating significant inhibition zones against microbial strains. These findings highlight the potential of irradiated citrus essential oil nano emulsions as natural bioactive agents with enhanced stability and efficacy. Their strong antioxidant and antimicrobial properties suggest their suitability as natural preservatives for food safety and other applications, providing a sustainable approach to utilizing citrus peel waste.

Keywords: Orange and lemon essential oil, Nanoemulsion, Gamma radiation, Antimicrobial, Antioxidant.

1. Introduction

Essential oils are natural extracts with volatile compounds and stimulating odors, produced as secondary metabolites of aromatic plants, are well known for their antioxidant [1], anti-inflammatory [2], antimicrobial activities [3], and can be extracted from leaves, seeds, or fruit peel, is produced annually in the world at around 16,000 tons [1, 4, 5]. Orange and lemon EOs, primarily D-Limonene and linalool, are highly sought after by-products of citrus processing, exhibiting antioxidant, anti-inflammatory, and antibacterial properties (against several varieties of bacteria, molds and yeasts, and viruses), crucial in food chemistry, pharmaceutics, and cosmetics [6-9]. For instance, C. limon is cultivated for its alkaloids, which have antibacterial properties against major bacterial strains like (Escherichia coli, Staphylococcus aureus, and Salmonella spp.) [10, 11].

Citrus essential oil and their nano-emulsions constituents (essential oil, water and Tween 80) are environmentally friendly additives used in food, household detergents, sanitizers, cosmetics, and drinks due to their minimal opacity and sub-cellular scale, ranging between 20 and 200 nm, and are generally regarded as safe (GRAS) by the FDA and the European Union [12-21].

Food irradiation technology is accepted in over 50 countries, including Egypt, and is currently used to irradiate over 60 products globally. Gamma irradiation, a high-energy gamma ray produced by radioactive sources like cobalt-60 or cesium-137, is used for sterilization, food preservation, and radiation therapy [22-24]. "The safe use of γ-irradiation is regulated by the International Atomic Energy Agency and the U.S. Food and Drug Administration; however, it continues to be a promising method for the sanitization and evaluation of aromatic and medicinal plants [25, 26].

On the other hand, Citrus EOs' insolubility in water, volatility, low stability, and environmental sensitivity hinder their widespread use. Encapsulation in emulsions reduces hydrophobicity, but conventional emulsions are unstable and cause component separation [27, 28]. These problems can be addressed through nano emulsions (NEs) formulated using advanced nanotechnology, Nano emulsions are a class of emulsions with smaller droplet sizes from (20–200 nm) and have greatly better stability against creaming, flocculation, sedimentation and Ostwald ripening than convention emulsions [29-34].

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Nanoemulsions can act as a barrier against bacteria, fungi, and viruses by selectively binding their transparent or semi-transparent particles to the cell wall of prokaryotic cells, leading to destabilization [17, 35]. Moreover, the small droplet size enhances the material's bioactivity against bacteria by facilitating penetration of the cell membrane, destabilizing lipid bilayers, disrupting cellular functions, and ultimately leading to cell death [7, 36].

Therefore, researchers are developing nano emulsions to enhance stability and biological activities of essential oils, specifically for food processing and preservation purposes [37, 38]. The necessity of change from synthetic food additives to natural ones qualified citrus EO as a possible natural additive in the food and cosmetic industry [39, 40]. The world's nanotechnology manufacturing might value 80 billion US dollars and might be reaching 17% growing rate by 2024. Consequently, the potential benefits and incomes are huge in this developing market [41]. So, the study aimed to prepare and characterize nano-emulsions containing orange and lemon peels essential oils, using ultrasonic homogenizer. The nano-emulsions were evaluated for stability, antioxidant, and antimicrobial activities, and using to improve their applications in food as natural additive and cosmetics.

2. Material and Methods

2.1. Raw material

Lemon (Citrus lemon) and orange (Citrus sinensis) fruits were purchased from the Belbies City El-Sharqia governorate from local market The orange and lemon fruits were meticulously hand-cleaned to remove any trash. The peels were cut into tiny pieces, detached from the endocarp, sorted into appropriate specimens, and then put in plastic bags [1].

2.2. Chemicals and reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), (Produced by Sigma Corporation, St. Louis, MO, USA), sodium phosphate buffer, potassium ferricyanide, trichloroacetic acid and Tween 80, were bought from Merck Chemicals Ltd. (BHT) The synthetic antioxidant butylated hydroxytoluene (purity 99.9%) and tri-chloroacetic acid (TCA) were bought from local chemical companies. El-Gomhoria Company for Chemicals and Drugs, Cairo, Egypt, was the source of the analytical-grade chemicals and solvents.

2.3. Microbial strains

Two negative strains of *Salmonella typhimurium* (ATCC 98031) and *Escherichia coli* (ATCC 35218), as well as two Gram-positive strains of *Staphylococcus aureus* (ATCC 20231) and *Bacillus subtilis* (ATCC 9372). were obtained from the Egyptian Microbial Culture Collection (EMCC) at the Faculty of Agriculture, Ain Shams University, Cairo, Egypt. While, pathogenic fungus *Aspergillus brasilinesis* (ATCC 16404) and Candida albicans (ATCC 10231) were purchased from the Regional Center for Mycology and Biotechnology, Faculty of Science, El-Azhar University, Cairo, Egypt.

2.4. Extraction and preparation of essential oils

Following the orange and lemon fruits' washing and peeling, the essential oils were extracted and separated over the course of three to four hours using the water distillation method. Essential oils were separated by method which used a Clevenger apparatus [1] and [2]. After being extracted and dehydrated over anhydrous sodium sulphate, the volatile oil was kept in a deep freezer until it could be analyzed.

2.5. Irradiation treatment of essential oils

Gamma rays at doses of 0, 2, and 4 kGy were used to irradiate orange and lemon essential oils. A 60Co Russian gamma chamber (dose rate: 395.1 Gy/h) was used for all irradiation procedures at the Cyclotron Project, Nuclear Research Centre, Atomic Energy Authority, Egypt.

2.6. Preparation of Nano-emulsion

Nano-emulsion was formulated using citrus peels essential oils, non-ionic surfactant Tween 80 and water. Concentration of citrus peels essential oils (6% v/v) was fixed for the emulsion formulation. Tween 80 (2 v/v%) was dissolved in double-distilled water at room temperature and stirred magnetically for 10 minutes to obtain a homogeneous solution. The essential oil was added gradually and mixed using a magnetic stirrer (IKA-COMBIMAG RET, power: 620W, 220V and 50Hz; JANKE&KUNKEL GMBH&CO.KG, GERMANY). The obtained crude emulsion was subjected to sonication using a 25 kHz ultrasonic homogenizer (UCD-950, max power: 1000 watt/220V; BIOBASE BIODUSTRY SHANDONF CO., LTD, CHINA). Then, the formulated nano-emulsion was characterized. The sonication time was fixed for 10 minutes [43, 44].

2.7. Characterization of nano-emulsions

2.7.1. Particle size, poly dispersity index (PDI) and Zetasizer (Malvern) measurements

The droplet size distribution (volume-based analysis) and PDI of nanoemulsions were determined using dynamic light scattering (DLS) and analyzed with a Zetasizer (Malvern). This instrumentation was used to study the surface charge of the particles using (Zetasizer Ver. 6.32 Nano Series (HT), Nano ZS Malvern Instruments Ltd, UK) at room temperature. Samples were diluted to 10% with deionized water before analysis to minimize multiple scattering effects. Emulsion droplet size was determined as the mean diameter (nm) based on the average of three measurements [45].

2.7.2. Fourier transforms infrared spectroscopy (FTIR)

FTIR analysis were performed to determine the effective chemical groups that might be involved during the manufacture of nanoemulsions or be in charge of stabilizing them and concluding the functional group change as a result of the reduction process. FTIR was done using Agilent Cary 630 FTIR spectrometer by the use of an ATR sampling module. The wavenumber range was 4000–650 cm^{-1}, utilizing KBr disks prepared by mixing 100 mg of KBr with 100 µL of the tested sample and drying at 40°C [46]. Disks were formed by grinding 10 mg of KBr, pressing it in a die at 10 tons for 1 minute [36].

2.7.3. Transmission electron microscope (TEM)

The morphology of nanoemulsion droplets was examined using transmission electron microscopy (TEM) (JEOL JEM-1400 CX, Shimadzu Co., Tokyo, Japan) at an acceleration voltage of 80 kV. A 5 µL drop of diluted nanoemulsion was placed onto a 300-mesh copper grid with a carbon film and left to dry at room temperature for 48 hours, following the method of Moradi and Barati [44].

2.8. Determination antioxidant activity of citrus peels essential oils nano-emulsions

2.8.1. Radical scavenging assay (DPPH%)

A modified form of the Brand-Williams et al. [47] was used to examination the antioxidant properties. Antioxidant activity was assessed using the 2,2-diphenyl-picrylhydrazyl (DPPH) free radical assay, where antioxidants reacted with the stable radical in a methanol solution. The reduction in absorbance at 515 nm was observed alongside the discoloration of DPPH radicals, DPPH absorbs in its radical form; however, absorption is eliminated when an antioxidant species reduces the compound. A 6×10–5 moll/l of DPPH solution (2.4 mg of DPPH in 100 ml of methanol), (0.1mL) of essential oil was added to 3.9 ml of a 6 × 10-5 mol/L methanol DPPH e solution. After giving the mixture a good shake, it was left at room temperature for half an hour and kept out of the light according to [16, 21, 48]. A spectrophotometer was used to measure absorbance. Additionally, methanol was used as the blank.

Antioxidant activity (Inhibition)% = (Acontrol– Asample)/Acontrol×100

Where:

A control is the absorbance of the control reaction (absorbance of DPPH solution) and A sample is the absorbance (of the essential oil with DPPH solution). Samples were examined in triplicate.

2.8.2. FRAP assay

The examine was carried out according to the method of [49] with slight modifications to be carried out in micro plates, A freshly prepared TPTZ reagent was composed of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 (v/v/v) ratio. For analysis, 190 µL of the reagent was mixed with 10 µL of the sample in a 96-well plate (n=3) and incubated at room temperature in the dark for 30 minutes. The resulting blue color was measured at 593 nm. The ferric reducing ability of the samples was expressed as µM TE/mg sample using a linear regression equation derived from the Trolox calibration curve [21].

$$y = 0.0019x + 0.0874 \qquad R^2 = 0.9985$$

2.9. Evaluating the antimicrobial activity of citrus peels essential oils nanoemulsions

Antimicrobial properties of orange and lemon peel essential oils were investigated in vitro. by the agar well diffusion method with a few modifications [50, 51]. Mueller-Hinton sterile agar for an antibacterial examination and sterilized potato dextrose agar (PDA) for an antifungal examination. A cork borer (8 mm) was burned and used to bore one central well in each plate, which contained 50 µl (for an antibacterial test) and 100 µl for an antifungal test). The plates were saved at 7°C for 3 h to let the oil diffusion and then incubated at 37°C for 24 h (for bacterial growth) and 28°C for 72 h (for fungal growth) [13, 21]. The inhibitory zone was measured in millimeters following the incubation period. Every experiment was carried out three times.

2.10. Statistical analysis

Data were evaluated with SPSS analytical software version 18.0 (SPSS Inc., Illinois, USA, 2009) [52]. The data performed one-way analysis of variance (ANOVA), followed by the Duncan test for post-hoc mean comparison. Levels of significance were determined using a 95% confidence level ($p < 0.05$). For each independent analysis, three duplicates were performed, and the data was presented as mean ± standard diffiation (±SD).

3. Results and discussion

3.1. Particle size, poly dispersity index (PDI) and Zetasizer (Malvern) measurements

The study confirmed the quality of nanoemulsion by measuring its average droplet size and droplet size distribution using dynamic light scattering analysis and zetasizer. as shown in (Figures 1 & 2). Data showed that the mean particle size of the orange oil nanoemulsions for 0, 2, 4 KGy were 46.58, 29.92 and 47.12 nm respectively, with a poly dispersity index (PDI)

0.237, 0.268 and 0.283 respectively. While lemon oil nanoemulsions for 0, 2, 4 KGy were 17.6, 64.81 and 25.03 nm respectively, with a poly dispersity index (PDI) 0.378, 0.396 and 0.583 respectively.

International standards organizations state that PDI values >0.7 are more indicative of a broad range of particle sizes, whereas values <0.5 are more common for monodisperse samples [53, 54]. PDI indicates the uniformity of droplet size distribution in the nano-emulsion. All the nano-emulsions in the current study had low poly dispersity index values ranged from (0.237 to 0.583) which reflects the overall stability and uniformity of the formulation. The incorporation of OEO and LEO nano emulsions resulted in PDI values less than 0.5, indicating their stability in a monodisperse state. Particularly, the sample nano-emulsions showed the highest stability among the emulsions, with a zeta potential having a height negative charge values, a PDI less than 0.5, and smaller particle sizes [55].

Furthermore, the statistics show a narrow spectrum of sizes. Similarly, several scientists reported comparable droplet sizes in nano- emulsions [56].

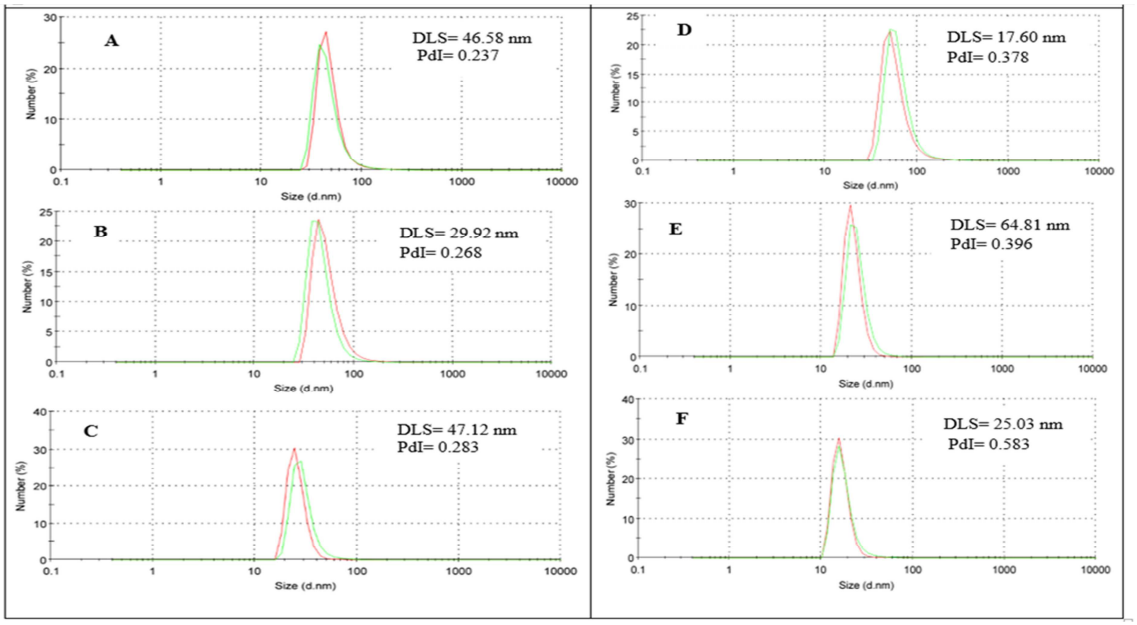

Figure 1: DLS and PDI analysis: (A, B and C):orange nanoemulsion; (D, E and F): lemon nanoemulsion at doses 0, 2 and 4 kGy respectively.

Furthermore, the range of the zeta potential is (-7.58 to -18.8 mV and -3.9 to -8.56 mV) for orange and lemon nano-emulsions, respectively. These numbers show how stable the emulsion is against sedimentation and creaming [38, 57, 58]. This results are in agreement with [59, 60] how reported that zeta potential value of *Citrus sinensis* L. nanoemulsions was −16.31 ± 2.54 mV. Similarly, agreement with [53] who found that the mean particle size of the nanoemulsions for (orange and tangerine peel oils) is certainly ultra-fine (<100 nm) and ranged from 29.41 to 66.41 nm with zeta potential values ranging from -14.27 to -26.74 mV, representing stability among 81.44% and 99.26%.

There was no change in the zeta potential when the concentration of the emulsifier increased [31]. The zeta potentials in all of the data sets utilized in this study have negative charges between -3.9 and -18.8 mV, which is still within the stable range and may help to avoid flocculation and coagulation. The results are in line with [55, 61]. All orange nano-emulsion samples showed higher anionic content than lemon nano-emulsion samples, according to the results of the current investigation. These results also concur with [53, 62], who reported that orange nano-emulsion had a stronger anionic zeta potential than lemon and cinnamon nano-emulsions.

Also, our results in this investigation are similar to [31] who reported that the mean particle size and zeta potential for *Citrus aurantium* L. var. essential oil were (21.52 nm and –9.82 mV) respectively. Likewise, the data presented in this study agree with [16] who found that the value for zeta potential was (-10.4 mV) for CEO-NEs, which shows moderate stability.

The zeta potential is also crucial for confirming the physical stability of emulsions; emulsions with a higher zeta potential, whether positive or negative, are thought to be more stable, according to [63]. In this regard, the orange oil nano-emulsion exhibited the highest zeta potential and the best stability with the smallest particle size and PDI [53], and the results of our investigation corroborate these conclusions.

Surfactants known as emulsifiers alter interfacial tension and increase interfacial flexibility. Consequently, the production of emulsions with small particles is more significantly influenced by the emulsifier [64]. Thus, little emulsifier amount leads to increased nano-emulsion particle size due to aggregation. However, sufficient emulsifiers decrease interfacial tension and stop aggregation, preserving the nano-emulsion's particle size within a suitable range [31]. Smaller particles can more efficiently penetrate microbial cell walls, disrupt essential functions, induce cell breakdown, and release intracellular contents [53].

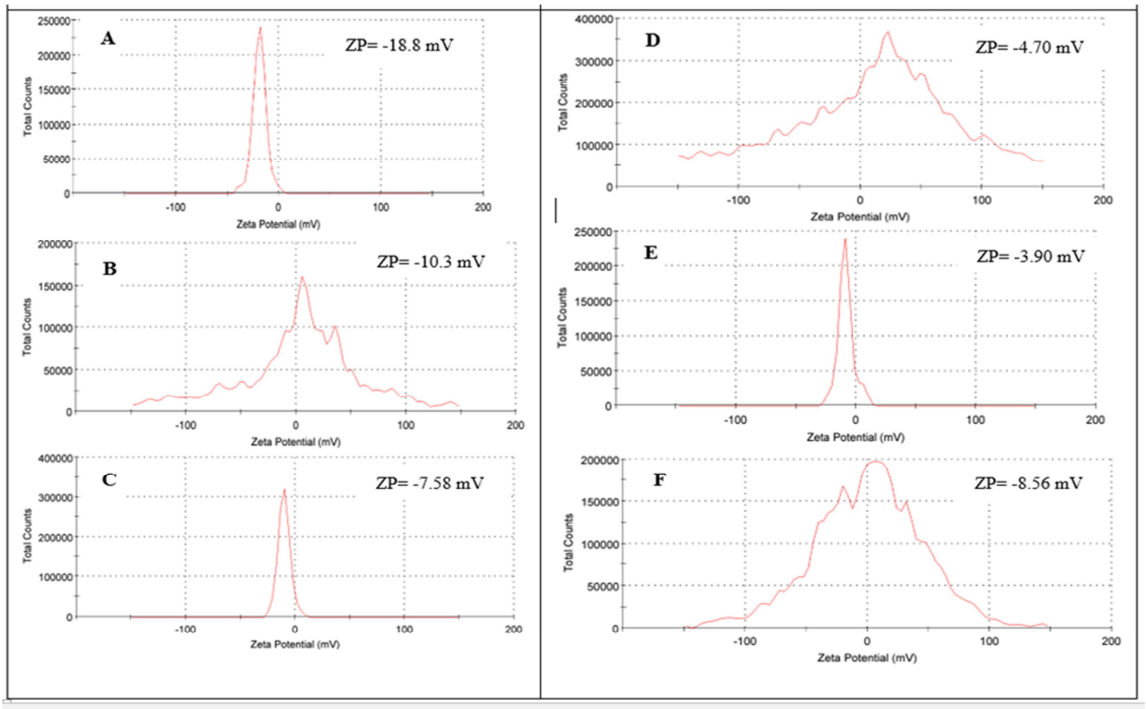

Figure 2: Zetapotential analysis: (A, B and C):orange nanoemulsion; (D, E and F): lemon nanoemulsion at doses 0, 2 and 4 kGy respectively.

3.2. **Fourier transform infrared spectroscopy (FTIR)** The identification of functional groups has made extensive use of Fourier transform infrared spectroscopy (FT-IR) [65, 66]. FTIR peaks for lemon and orange nanoemulsions were displayed in (Figure 3). The analysis indicated that the FTIR patterns of both lemon and orange nanoemulsions showed notable similarities and these results are consistent with [67]. The peaks observed at 3332 cm^{-1}, 2922 cm^{-1}, 1645 cm^{-1}, 1438 cm^{-1}, and 1088 cm^{-1} are likely attributed to the following functional groups: O-H stretching, C-H stretching (alkyl groups), C=C stretching, C-H bending (alkenes), and C-O stretching (esters or alcohols), respectively [68, 69]. Furthermore, the study revealed that exposure to gamma rays, with doses ranging from 0 to 4 kGy, resulted in no significant effect on citrus essential oils nanoemulsion. Our finding data from FTIR result of citrus essential oil agree with other investigation were gotten by. Our citrus essential oil FTIR results are consistent with those of prior studies obtained by [70-74].

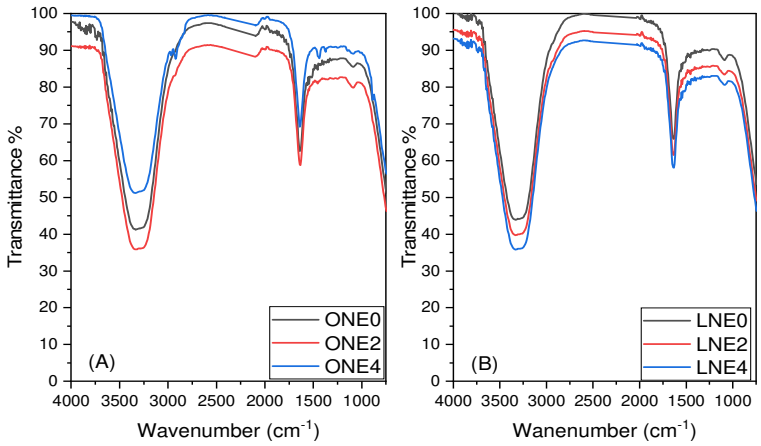

Figure 3: FTIR for irradiated and non-irradiated (A) orange and (B) lemon essential oils nano-emulsion

3.3. Transmission electron microscope (TEM)

TEM analysis of CEO-NEs revealed that the spherical and uniform particle shape and also, confirmed the results of the particle size analysis using dynamic light scattering (DLS). As shown in (Figure 4) the TEM micrographs in current study displayed that the nanoparticles were spherical, uniformly distributed, discrete, non-aggregated, and exhibited varying average diameters in nanometers. The average size of the droplets was around diameter of (17-65 nm) based on images for orange and lemon peels essential oils, and agreement with the results of DLS measurement.

The nanoemulsion particle size ranged from 10 to 100 nm (Figure 4), aligning with particle size measurements and confirming that the prepared emulsions were true nanoemulsions. This is results was agreeing with DLS analysis. Also, data obtained in this current investigation are similar to [13, 75]. The size of droplets in a nano-emulsion plays a crucial role in determining their physicochemical and functional characteristics, including its visual attributes, consistency, storage stability, and behavior inside the gastrointestinal system [16, 76]. TEM software adjusted the difference between background and particles and calculated the diameter of rounded nanoparticles automatically [75].

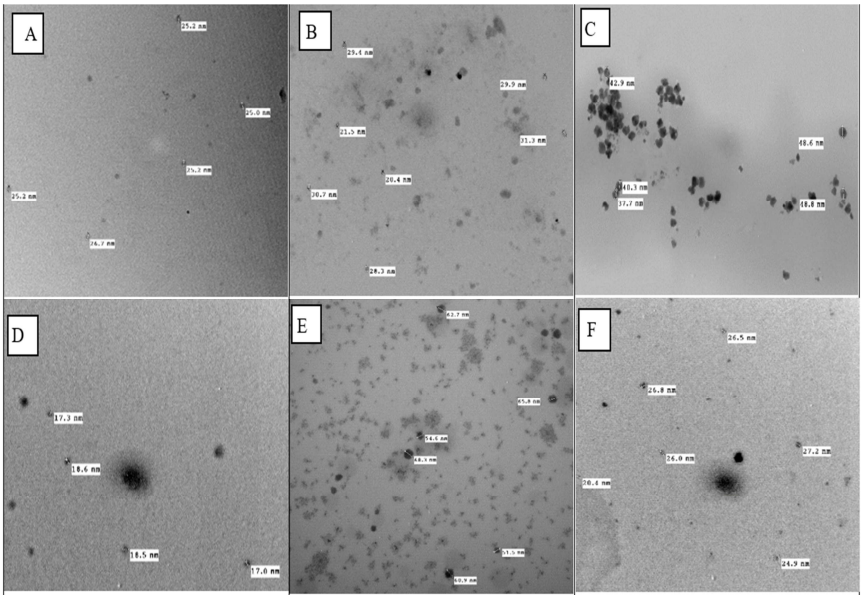

Figure 4: TEM image: (A, B and C):orange nanoemulsion; (D, E and F): lemon nanoemulsion at doses 0, 2 and 4 kGy respectively.

3.4. Antioxidant Activity

3.4.1. Determination of radical scavenging activity (DPPH%) and FRAP assays

The antioxidant activity of the two CEO-NE types by the DPPH radical-scavenging activity was greatly enhanced by raising the gamma irradiation dose from 17.3 to 19.3% for orange and from 15.5 to 21.9% for lemon nano emulsion, respectively, as shown in (Table 1). Additionally, depending on the irradiation level of the samples, the antioxidant activity of CEO-NEs using the FRAP approach varied from 764.5 to 840.7 µM TE/mg for orange and 1011.9 to 1138.9 µM TE/mg for lemon nanoemulsion. As the radiation dose grew, the antioxidant activity of the nanoemulsion essential oil, as demonstrated by DPPH and FRAP, typically increased as well, peaking at dose levels of 4 kGy, as indicated in Table 1. The ability to convert the stable DPPH free radical to its reduced form, DPPH-H, is known as DPPH scavenging activity [77]. Also, the results in the present investigation are similar to [78] who reported that the in vitro antioxidant capacity of peels from four citrus species (Lima orange, Pera orange, Tahiti lime, Sweet lime, and Ponkan mandarin) was higher than that of pulps in the ferric reducing antioxidant power (FRAP) assay [79], and the (FRAP) assay values was ranged from (744.0 to 3897.9 µ mol of trolox equivalent/100 g of FW) for all tested citrus. Also, similar to the results in the present study, were obtained by [53].

The obtained results correspond with earlier research conducted by [60, 80], who found that the DPPH inhibition activity (%) for Citrus sinensis peel essential oil nano emulsion were varied from (12.956 to 18.213 %). Also, our findings from the most recent study are similar to those of earlier studies [81], who found that the antioxidant activity of lemon peel oil nanoemulsion and encapsulated form ranged from 12 to 20% as determined by DPPH% analysis.

All Citrus sinensis peel essential oil nano emulsion samples were showed antioxidant activity, which may have been caused by the presence of phenolic compounds in citrus sinensis essential oil (CSEO), which accelerated the breakdown of the free radical chain reaction [21, 82, 83]. Monoterpene-rich essential oil nano-emulsions are natural antioxidants that shield food items from damaging oxidative impacts, according to [16, 84].

One of the main constituents of the essential oil, D-limonene, has antioxidant properties and may be responsible for the high levels of naturally occurring antioxidants in the oil in the current study in terms of free radical scavenging [16]. Additional studies have revealed a correlation between the chemical structure and anti-oxidative properties of CEOs loaded nano-emulsions [4, 16, 85, 86].

Previous DPPH assay results showed that nanoemulsions exhibited higher scavenging activity than pure essential oils. At an EO concentration of 0.12 mg/mL, activity increased from 30.5% (pure EO) to 51.6% (nanoemulsified EO). When the EO concentration was quadrupled, antioxidant activity further increased from 44.3% (pure EO) to 72.4% (nanoemulsified EO) [16]. These outcomes are near to the antioxidant capacity of citrus Eos nano-emulsion in our study.

Depending on the dose, length of exposure, extraction technique, and sample, radiation can boost antioxidant activity. After irradiation, increased antioxidant activity caused the polysaccharide chain to break apart, producing hydroxyl groups and low molecular weight molecules that in turn decreased hydrogen intermolecular connections. Additionally, irradiation breaks down and activates the enzyme phenylalanine ammonia-lyase.

Table 1: Antioxidant properties of citrus peels essential oils nanoemulsions

	DPPH %		FRAP(μM TE/mg)	
BHT(200 mg/L)	86.2 ± 1.312^{A}		- -	
Irradiation dose	ONE	LNE	ONE	LNE
0 kGy	17.7 ± 0.506^{D}	15.5 ± 1.967^{D}	764.5 ± 5.263^{C}	1011.9 ± 5.263^{C}
2 kGy	19.0 ± 0.615^{C}	18.4 ± 0.556^{C}	810.1 ± 3.059^{B}	1027.7 ± 5.263^{B}
4 kGy	21.6 ± 0.903^{B}	22.9 ± 0.759^{B}	840.7 ± 4.849^{A}	1138.9 ± 4.717^{A}

ONE: Orange nanoemulsion, LNE: Lemon nanoemulsion, calculated mean is for triplicate measurements ± SD; values with different superscripts in the same column are considered statistically different ($p > 0.05$).

3.5. Antimicrobial activity

The study investigates the antimicrobial efficacy of orange and lemon essential oils nanoemulsions (NE) at different irradiation doses (0, 2, and 4 kGy) against Gram-positive bacteria, Gram-negative bacteria, and fungi as exposed in (Table 2). The results demonstrate that increasing irradiation doses generally enhance the antimicrobial activity, particularly at 4 kGy. Lemon NE consistently showed higher inhibition zones compared to orange NE across all tested microorganisms. Notably, at 4 kGy, lemon NE exhibited the strongest inhibitory effects, especially against Gram-negative bacteria and fungi, indicating a significant enhancement in antimicrobial potency. This enhancement can be attributed to radiation-induced modifications in the chemical composition of the essential oils, potentially increasing the availability of active antimicrobial compounds. Overall, gamma irradiation appears to improve the natural antimicrobial properties of citrus essential oils, suggesting potential for effective microbial control.

The results of this study also mirrored those of [87], who discovered that lemon oil had potent antibacterial action against S. aureus, with inhibition zones spanning from 25 mm to 28.33 mm. Also, orange essential oils showed high action against E. coli, with inhibition zone diameters ranging from 17.67 mm to 26.67 mm. likewise, our findings from the most recent study are similar to those of earlier studies [81], who found that the antimicrobial activity for lemon oil nanoemulsion and its encapsulation increased by increasing the percentage of the oil with an inhibition zone varying from 30 to 44 against Staphylococcus aureus and 10 to 18 mm against Escherichia coli, respectively. Likewise, our results are consistent with previous research that found that the inhibition zone of Citrus spp. for E. coli ranged between 5.8 and 21.0 mm [88].

The activity of the inhibition zone varied: zones larger than 20 mm were considered very active, zones between 12 and 20 mm were considered mediumly active, and zones smaller than 12 mm were considered somewhat active [21, 55, 89]. In contrast to orange peel essential oils NEs, which displayed moderate to strong antibacterial activity, the irradiated and unirradiated lemon peel essential oils NEs was considered the strongest antimicrobial agent.

Our data are in agreement with [60], who demonstrated that CSEO nano-emulsions were more effective than CSEO emulsions at penetrating the cell wall of Gram-negative bacteria. Gram-positive bacteria lack an outer membrane, in contrast to Gram-negative bacteria [85]. According to recent research, several gram-positive and gram-negative bacteria are sensitive to the effects of sweet orange and lemon oil [90].

Likewise, results indicate that the citrus essential oil nanoemulsion exhibits a notable antifungal activity against both Candida albicans and Aspergillus brasilinesis. Citrus nanoemulsion's, both irradiated and non-irradiated, had antifungal activity. The highest inhibition zone was at gamma irradiation dose 4 kGy. The diameter of the inhibition zone for Candida albicans (varying from 18.7 to 30.2 mm for orange and 22.4 to 36.8 mm for lemon) is greater than that for Aspergillus brasilinesis (varying from 10.7 to 21 mm for orange and 21.6 to 31.8 mm for lemon), suggesting that Candida albicans is more susceptible to the compounds present in the citrus essential oil nanoemulsion.

The data presented in this work agreed with [13, 81, 87] who reported that the antifungal activity of citrus essential oils and their nanoemulsions was assessed, and the inhibition zones varying from 19.66±3.15 mm to 41.66±2.88 mm against C. albicans. nanoemulsions was assessed, and the inhibition zones varying from 19.66±3.15 mm to 41.66±2.88 mm against C. albicans. The data presented in this work supported their findings. Interestingly, it was shown that greater dosages considerably enhanced the effectiveness of the C. limon nano-emulsion. Essential oils can both stop the growth of fungi and regulate the production of mycotoxin, which ultimately leads to death [91, 92].

In agreement with [93-95], citrus essential oil, particularly lemon and peel oils, were significant antimicrobial properties against fungi like Aspergillus brasiliensis and Candida albicans due to their rich in terpenes, such as limonene, linalool, γ-terpinene and terpinen-4-ol, which are considered to be a natural antifungal properties due to their non-polar chemical structures, are considered natural antifungal agents due to their non-polar chemical structures, characterized by hydrophobic and lipophilic properties that enhance interaction with fungal cell membrane components. Essential oils penetrate the cell wall and cytoplasmic membrane, disrupting the structural integrity of polysaccharides, fatty acids, and phospholipids. Additionally, they interfere with enzymes and membrane proteins involved in ergosterol biosynthesis, leading to proton efflux and cellular destabilization [13, 96].

Moreover, the antimicrobial activity of plant essential oils can be significantly enhanced by their submicron droplet size, facilitating easier penetration through microbial membranes, leading to cell content leakage and eventual cell death. This effect may be attributed to the hydrophobic nature of nano-emulsions, which influences the key components of citrus essential oils [13, 16, 32]. Citrus peels essential oils nanoemulsions enhance antifungal activity due to increased surface area and better penetration capabilities. So, this has been shown to improve the effectiveness against Candida albicans and Aspergillus brasiliensis [87, 97].

The stability values were mostly determined by the zeta value, and the stability value of this oil nano-emulsion was the greatest. By influencing fungal metabolites, these values enhance the emulsion's capacity to lower toxins [98]. Because of their small size, nano-encapsulated EOs have a larger surface area, which helps them interact with microbial cell membranes more effectively and at the right dosages [99].

Table 2: Antimicrobial activity of citrus peels essential oils nano emulsions

	Inhibition Zone (mm)											
	Gram (+) bacteria				Gram (-) bacteria							
	Staphylococcus aureus		*Bacillus Subtilis*		*Escherichia coli*		*Salmonella typhimurium*		*Candida albicans*		*Aspergillus brasiliensis*	
Irradiation dose	Orange (NE)	Lemon (NE)	Orange (NE)	Lemon (NE)	Orange (NE)	Lemon (NE)	Orange (NE)	Lemon (NE)	Orange (NE)	Lemon (NE)	Orange (NE)	Lemon (NE)
0 kGy	14.0± 0.81^{B}	33.2± 4.42^{B}	14.5± 0.5^{B}	26.0± 0.8^{C}	15.5± 1.29^{B}	21.2± 2.50^{C}	16.5± 0.57^{A}	23.0± 1.82^{C}	18.7± 0.289^{C}	22.4± 1.600^{C}	10.7± 0.289^{C}	21.5± 0.503^{C}
2 kGy	17.2± 0.50^{A}	30.5± 0.57^{B}	15.5± 1.2^{AB}	33.0± 0.82^{B}	17.0± 0.87^{B}	29.0± 0.81^{B}	16.5± 1.29^{A}	31.7± 2.36^{B}	25.2± 1.756^{B}	29.5± 1.323^{B}	15.1± 0.361^{B}	25.0± 1.000^{B}
4 kGy	16.5± 0.57^{A}	39.7± 1.25^{A}	17.5± 1.72^{A}	36.5± 2.3^{A}	19.2± 1.71^{A}	33.2± 1.50^{A}	15.7± 1.71^{A}	36.0± 1.41^{A}	30.2± 0.764^{A}	36.8± 1.258^{A}	21.0± 1.00^{A}	31.8± 1.607^{A}

Calculated mean is for triplicate measurements ± SD; values with different superscripts in the same column are considered statistically different ($p > 0.05$). NE: nanoemulsion.

Conclusion:

Ultrasonic emulsification can be used to create a nano-emulsion in a citrus-in-water system. Citrus nano-emulsions shown antibacterial and antioxidant qualities against fungus and bacteria. A promising technique for enhancing the stability, efficacy, and safety of these valuable chemicals is the combination of gamma irradiation and citrus essential oil nano-emulsion. Nano-emulsions' small droplet size facilitates improved penetration and interaction with target sites, and gamma irradiation increases their antimicrobial and antioxidant properties while extending their shelf life. In particular, 4 kGy demonstrated higher inhibition zones and antioxidant activity than other doses. This study's objective was to provide an overview of the characteristics of citrus essential oil nano-emulsions. The entire potential of this technique and its applications in a variety of industries, such as food flavoring, natural antibacterial and antioxidant agents, natural additives, agriculture, cosmetics, and healthcare, were revealed by further research.

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