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Research article

Effect of γ irradiation on the antibacterial activity of poly lactic acid films encapsulated with essential oils against some common food borne pathogens

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Abstract: The production of healthy, eco-friendly, sustainable, and active food packaging is necessary to replace the existing conventional synthetic packaging. The aim of this study is to develop new food packaging materials from polylactic acid (biodegradable polymer) encapsulated with volatile oils for the production of active packaging that has a functional effect on food borne pathogens (antibacterial properties) and to study the impact of gamma irradiation on the antimicrobial activity of these packages. Lemongrass essential oils (LGEO) and Cumin essential oils (CEO) and in combination (mix EOs), their incorporation complex into polylactic acid (PLA) films and the antibacterial activity of active PLA films before and after γ-irradiation (5 and 10 kGy) were studied against three important food pathogens; *Escherichia coli O157:H7*, *Listeria monocytogenes* and *Salmonella enteritidis*. Also, the mechanical and physical properties of the active films were evaluated. PLA/LGEO showed good stability with a suitable prolonged release of LGEO, resulting in improved antimicrobial activity compared to the PLA/CEO. Due to the synergistic effects of mix EOs, PLA/mix EOs films showed complete inactivation against tested bacteria, suggesting that the evolved PLA/mix EOs films have great potential for food active packaging applications, so PLA/mix EOs were selected for further analysis. Irradiation at dose 5 kGy has no significant effect on the antibacterial activity of PLA/EOs films, increasing the radiation dose to 10 kGy, resulted in a significant decrease in active PLA/EOs films antibacterial activity. The combination of lactic acid active packaging and 5 or less doses of gamma irradiation will have a synergistic effect against food borne pathogen, thus achieving the quality and safety of irradiated foods.

Keywords: antimicrobial packaging; food pathogens; polylactic acid; volatile oils; γ-irradiation

1. Introduction

Recently, active systems of food packaging have been developed by incorporating antimicrobial agents or chemical preservatives into the polymeric packaging film materials. The release of antimicrobial agents from the active packaging of foods to the food surface inhibits microbial contamination during storage [1,2]. Polymers have become commonly used in many applications, so it is difficult to imagine what the world would be without plastic. Production problems of polymer materials include its high costs as they are derived from fossil fuels. Also, customer demands for non-fossil alternatives have triggered research for developing alternative materials for food packaging from raw bio-materials [3]. Bioplastics (plastic polymers) are manufactured from renewal resources. Bioplastics became attractive alternatives for synthetic polymers as food packaging materials, due to their degradation by microbial enzymes (biodegradation), which minimize the accumulation of plastic waste and its environmental problems. The mass production of polymers materials gives the reduction of price. Nowadays, biopolymer can be prepared from agriculture waste that enhancement the competition in cost production with common petroleum-based polymer [4]. The expected growth of the bio-plastics market ranges from 20 and 25% up to 2020 [5]. Incorporation of additives, such as dyes, antimicrobials, antioxidants, flavors, and spices in film formulation, improve the ability of bio-plastic films to retard moisture, aromas, oxygen, and solute transport [6,7]. In response to market trends and consumer demands, the active packaging, especially food packaging, becomes an important consumer-friendly packaging [8,9]. Essential oils are concentrated natural extracts derived from plants were recognized as safe substances by Health and Human Services Public Health Services and several studies suggesting their applications in the food industry [10,11]. It has been reported that direct addition of plant essential oils to foodstuffs exert an antimicrobial effect [11] against pathogens and food contaminants [10].

Essential oils incorporation in bio-plastic films (active packaging) may be more effective than the direct use of antimicrobials in food [12]. Selective and gradual migration of antimicrobials, from the film surface towards the food surface, maintains high concentrations, reducing toxicity and prolonging the lifetime of agents during extended exposure on the surface of food [13,14]. The use of Poly-Lactic acid (PLA), which considers as one of the most promising biopolymers from renewable resources, in food packaging as bio-based material has already, received great attention. PLA is one of unique biopolymer based on eco-friendly production process for natural starch crops. It is also, biocompatible, and nontoxic instead of antimicrobial efficiency [15]. The production of PLA biopolymers from plant raw materials contributes to the reduction of CO_2 emissions, which has become one of the most important topics for the whole world. Numerous research activities, including the development of biodegradable, recyclable, smart, and bioactive food coatings and packaging materials, have been carried out for the development and improvement of new materials that can be used in the food sector [16,6].

The World Health Organization considers food irradiation important toward ensuring food safety and reducing food losses. Low-dose (<2 kGy) and medium-dose (between 1 and 10 kGy) irradiation applications are currently being investigated with food products such as poultry, meat and meat products [17].

Present day food processors prefer that food be prepackaged in the final packaging form before irradiation to prevent recontamination and to facilitate prompt shipment to market after irradiation. Lower dose levels (5–10 kGy gamma radiation) did not significantly change the mechanical and permeation properties as well as overall migration levels of commercial multilayer films [18]. 60 kGy gamma irradiation dose induced mechanical change but did not affect the thermal, and permeation properties of the film [19].

Hence, the main goal of this study is to develop new antimicrobial films based on the incorporation of volatile oils in polylactic acid films (PLA/mix EOs) to enhance the functional properties of antimicrobial films for food packaging applications. The effect of two specific γ irradiation target doses (5 and 10 kGy) on the antibacterial activity of the films was evaluated, and shed light upon finding a new technique between PLA/mix EOs and γ-irradiation to achieve the quality and safety of packaged foods.

2. Materials and methods

2.1. Materials

2.1.1. Media and chemicals

Brain Heart Infusion agar (BHIA), Brain Heart Infusion broth (BHIB), Plate Count Agar, Polymyxin-Acriflavin-Lithium chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) agar base, PALCAM selective supplements, and Xylose Lysine Deoxycholate agar (XLD) were obtained from (Sigma Chemicals, St. Louis, Missouri, USA). Cumin (*Cuminum cyminum*), Black seeds (*Nigella sativa*), Thyme (*Thymus vulgaris*), Coriander (*Coriandrum sativum*), Black pepper (*Piper nigrum L*), Chamomile (*Matricaria chamomilla*) and Lemongrass (*Cymbopogon flexuosus*) powder were purchased from local market in Cairo, Egypt.

L-lactic acid (LLA) was obtained from PURAC bioquimica (Barcelona, Spain) as a 90 wt per cent aqueous solution and was purified using a molecular distillation method to further reduce the water content before being used. Stannous octoate (tin (II) 2-ethylhexanoate) (Sn $(Oct)_2$) was from Aldrich Chemical Company (Germany). Chloroform, methanol, ethyl acetate, and tetrahydrofurane were purchased from El-Nasr Co. for Chemical Industries (Egypt) and used without any further purification. Polyethylene sheets and plastic weld units were received as a gift from Zepter Co. Sweden.

2.1.2. Bacterial strains

Fresh poultry meats and meats samples were obtained from different commercial suppliers. All samples were placed in sterilized plastic bags and placed in a cooler to be transported immediately to the laboratory. All samples were processed for isolation of their microbial load. The isolated bacteria were identified and stored in the culture collections of the Microbiology Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt.

Bacterial strains, selected from the stored isolates, used in this study were Gram-negative aerobic *Escherichia coli O157:H7*, *Salmonella enteritidis*, and Gram-positive aerobic *Listeria monocytogenes*. All strains were aerobically grown at 37 ℃ on BHI broth.

2.2. Methods

2.2.1. Synthesis of polymers

P-toluene sulfonic acid and stannous octoate (0.3 Wt%) were used as a catalyst for L-lactic acid (1.03 g) polymerization, stirred for 12 h at 150 ℃, then 20 mL acetone was added. The insoluble white precipitate (1.41 g) was collected by filtration and dried over several days under vacuum at 40 ℃.

2.2.2. Gel permission chromatography (GPC)

On water equipment with a quaternary pump controller Model 600 and a differential refractometer as the detector, the molecular weight was calculated using Gel Permitted Chromatography (GPC). To assess the molecular weight, two types of Waters Styragel HT 6E and HR 4E columns were used. According to the optimal operating conditions of each column, the system is run at a flow rate of 0.4 mL min^{-1}. The molecular weight is calculated in tetrahydrofurane according to polystyrene standards [20].

2.2.3. Scanning Electron Microscopy (SEM)

An SEM, Model JSM 5800 LV from Jeol Co., Japan was used. The SEM has a maximum magnification of 300,000 and a resolution of 3.5 nm. Measurements of both high and low vacuum can be carried out [21].

2.2.4. Transmission of water and oxygen

Water vapor transmission rate (WVTR) was measured using PERME-water vapor transmission rate analyzer, Labthink Co. All samples were investigated within test range: 0.001-50 g/m^2.day at 25 ℃ under humidity of 90%. In addition, Oxygen Transmission Rate (OTR) was measured with PERME-OX2/230 from Labthink Co., within measuring range from 0.01 to 6500 $cm^3/(m^2 \cdot day)$, at temperature of 25 ℃ and humidity 90% [22].

The Mass gain, % (water absorption), was calculated according to the following equation:

$$\text{Mass gain} = [(M_{wet} - M_{dry})/M_{dry}] \times 100 \quad (1)$$

2.2.5. Particle size distribution

Dynamic light scattering (DLS) instrument (PSS, Santa Barbara, CA, USA), using a HeNe laser line 632 nm as incident light with angel 90o and Zeta potential with an external angel 18.9o was used [23].

2.2.6. Essential oil extraction

Volatile oils from Cumin, Black seeds, Coriander, Black pepper, Lemongrass, Thyme and Chamomile were extracted by steam distillation for 3 h and stored at 4 ℃ until being used [24].

2.2.7. Determination of essential oils antibacterial activity by disc diffusion method

The antibacterial effect of extracted essential oils was screened using the disc diffusion technique [25] against the three above mentioned food borne pathogens with remarkable importance for food safety. On the surface of a BHIA plate previously inoculated with 200 μL of overnight bacterial culture (1×10^8 CFU/mL), a filter paper discs (Whatman No.1, 6 mm diameter) containing 15 μL of each essential oil were added. Fluclox (broad-spectrum antibiotic) disc (10 μg) was used as a positive control. The plates were incubated for 24 h at 37 ℃ and the diameter of the resulting inhibition zone was measured in millimetres.

2.2.8. Minimum inhibitory concentration (MIC) determination by well diffusion method

The minimum inhibitory concentration (MIC) of Lemongrass, Cumin essential oils, and their combination were determined using the agar well diffusion method on BHIA plates that seeded with the tested bacteria at inoculums 1×10^6 CFU/plate by spreading of 100 μL overnight culture. Wells (6 mm) were made with a sterile cork borer, and serial dilutions were applied for the essential oils with Dimethyl sulfoxide (DMSO) to obtain different concentrations (0.1%, 0.3%, 0.5%, 1%, 2%, and 4%). For each well, 20 μL of specific concentration was added and the same volume of DMSO was used as a control. After incubating at 37 ℃ for 24 h, the radius of the growth inhibition zone around the well was measured using a digital caliper. Results were measured after subtracting the radius of the well. The experiment was performed in triplicate [26].

2.2.9. Preparation of antimicrobial active packaging film

Polylactic acid solution was prepared with 5% weight in ethyl acetate over magnetic stirrer, overnight. The extract of lemon oil was used as stock with various concentrations namely, 0.25, 0.5 and 1g per 100g of polylactic acid. All mixtures were homogenized with ultrasonic homogenizer, Branson model, UK, at 80% amplitude for 5 min. Each concentration was used to spread manually one side of the polyethylene film using a coating rod [27]. Packaging bags were prepared with a fixed dimension 10 × 10 cm from the coated sheet and welded with Zepter plastic film welding unit.

2.2.10. Determination of antibacterial effects of irradiated and non-irradiated coated films

The inhibition zone assay on solid media was used to assess the antibacterial activity of (coated films with EOs) against selected bacterial strains. Twenty milliliters of molten BHIA were inoculated by 200 μL bacterial cultures (1×10^8 CFU/mL). Circular discs were cut from coated films PLA/LGEO, PLA/CEO and in combination (PLA/mix EOs) that prepared previously using a cutting well, and then divided into three groups; the first was not irradiated and considered the control group, while the second group and the third group were exposed to gamma irradiation at 5 kGy and 10 kGy dose levels, respectively. Irradiation was performed in the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt, at dose rate 2.519 kGy/h using the "Indian Gamma Chamber 4000 A" with a ^{60}Co source. Test discs were placed on the bacterial lawns and the plates were incubated for 24 h at 37 ℃. The plates were examined for "zone of inhibition" of the film discs [28].

2.2.11. Statistical analysis

Two-way analysis of variance (ANOVA) assessed the significance of the results. The analysis was performed using SAS software package version 9.1. All data were conducted in at least three replicates. The results were expressed as mean ± standard deviation and tests were carried out with probability limit of $P < 0.005$.

3. Results and discussion

3.1. Gel Permeation Chromatography (GPC)

Molar ratios of the prepared polymer resin were measured. The relatively low polydispersity was detected with a Unimodal chromatograph. PLA homopolymer was illustrated as PDI ≈ 1.3 with Mn ≈ 12100 Da and Mw ≈ 15700 Da which enhances its processing properties as an effective coating for packaging materials. Low polydispersity is an important value to polymeric packaging materials because it is related to deficiencies in a diversity of polymer chain length [29]. This leads to avoiding low molecular weight migration or oligomer transfer of packaging material to packed items.

3.2. Scanning Electron Microscopy (SEM)

PLA and PLA/essential oils samples were investigated by SEM microscope. The morphology of PLA before and after combining with the extracted essential oils can be directly examined by SEM (Figure 1). Topographic images showed that PLA was a kind of homogenous morphology. The roughness of the film surface increased with the presence of essential oils in PLA films. This indicated that the interaction between the essential oils was mostly homogenized with PLA polymer.

Combined PLA/LGEO, PLA/CEO and PLA/mix EOs with different ratios combined based on the weight of the extracted essential oils. As shown in Figure 1 (A) and (B), it was clearly observed that the fracture surface of raw PLA was smooth and clear while PLA/LGEO fracture surface was rougher and has more detailed surface features compared with raw PLA as mentioned in previous studied [30]. There was regular topographic behavior on the fracture surface, which phases were visualized in PLA/CEO and PLA samples (Figure 1 (C) and (D)). At displayed the part toughness, many rougher fracture surfaces, and a greater number of dispersed the presence of different essential oils, combining cumin with lemongrass content in PLA matrix, increasingly homogeneous dispersion of essential oils.

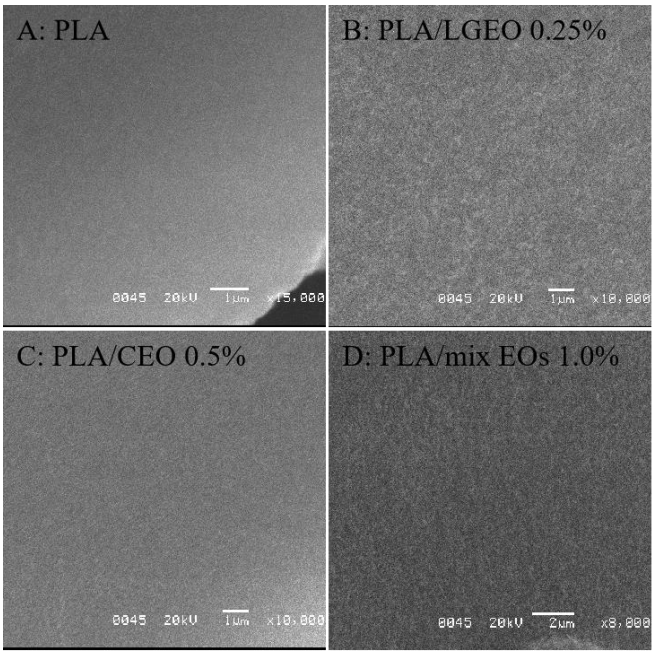


Figure 1. SEM topographic images for PLA film and PLA PLA films with lemongrass and cumin.

3.3. Water and oxygen transmission rate

The barrier performance of plastic is assigned to describe the ability of plastic film or enclosures to barrier the transmission of small molecule gas (such as CO_2, O_2, and N_2), fragrance, water vapor, and other organic solvents. The lower the rate of transmission, the greater is the efficiency of the barrier.

Films of PLA are well known as a "Screen Door" to Oxygen by its high transmission rate, it has a pretty good WVTR and it is good for OTR. The film was investigated after coating with PLA nano-flexible polymer which enhanced the barrier properties as shown in Table 1. The OTR of the coated film was reduced by 50–60 percent compared to the uncoated flexible packaging film. In addition, The WVTR was reduced by 40–80 percent of the uncoated film. The oil will be enhancement the elasticity of the film as a plasticizer which creates a dynamic elastic polymer chain under pressure of water vapor or oxygen. That is cause low permission of OTR and WVTR [31]. A modified LLDPE film with PLA coating will open the door for new applications to this packaging material with its unique barrier properties.

Table 1. Barrier characteristics of commercial packaging material linear low-density polyethylene (LLDPE) coated with PLA/LGEO, CEO or mix EOs coating.

Coating	LO/C Ratio wt%	OTR ($cm^3/(m^2 \cdot day)$)	WVTR ($g/(m^2 \cdot day)$)
LLDPE	-	2400	18.1
LLDPE/PLA	-	2900	17.3
LLDPE/PLA/LGEO [1]	0.25	3100	11.4
LLDPE/PLA/LGEO [2]	0.50	3400	10.1

Continued on next page

Coating	LO/C Ratio wt%	OTR ($cm^3/(m^2 \cdot day)$)	WVTR ($g/(m^2 \cdot day)$)
LLDPE/ PLA/LGEO [3]	1.0	3500	9.7
LLDPE/PLA/CEO [1]	0.25	2950	12.4
LLDPE/PLA/CEO [2]	0.50	3300	10.9
LLDPE/PLA/CEO [3]	1.0	3450	10.1
LLDPE/PLA/(mix EOs) [1]	0.25	3000	12.1
LLDPE/PLA/(mix EOs) [2]	0.50	3300	10.7
LLDPE/PLA/(mix EOs) [3]	1.0	3550	9.9

Note: PolyLactic Acid (PLA), PLA/Lemongrass essential oil (PLA/LGEO), PLA/Cumin essential oil (PLA/CEO), PLA/ mix EOs (PLA/mix of EOs), 1= 0.25 wt%, 2=0.50 wt%, 3=1.0 wt%.

3.4. Particle size distribution

For dilute solutions, DLS can be used as the main tool for understanding and validating polymer dynamics based models. This allows determining the size and hydrodynamic radius of polymer molecules in solution [32].

The particle size distribution of PLA which dispersed in aqueous medium results in a relatively homogenous particle diameter measurement distribution. Low polydispersity, narrow distribution of particle size, of PLA indicated to well-defined polymer chains (Figure 2).

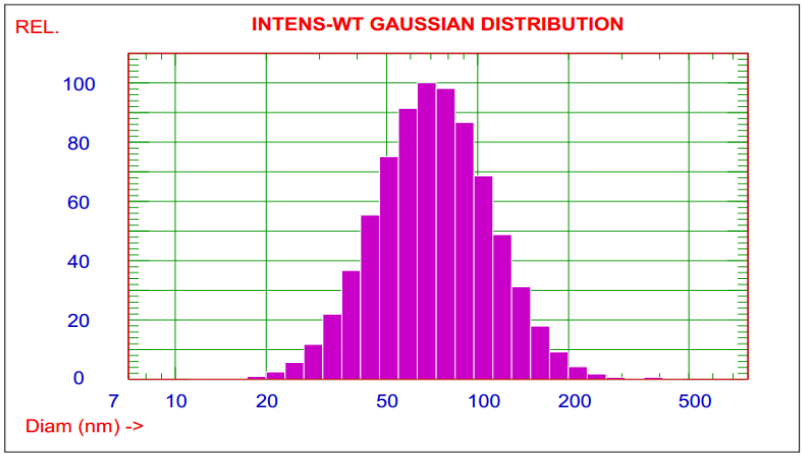


Figure. 2. Particle size distribution of PCL, gelatin and PCL/Gel blends with different ratios.

As shown in Table 2, synthesized PLA has a nano-particle size with a diameter of ≈ 78 nm. In addition, the standard deviation of size distribution pointed to uni-model behavior of nano-PLA. The variance of polydispersity index confirmed the well-defined character of PLA with value of ≈ 0.14. The polymers chains were self-assemblied in the aqueous medium to form ordering micelles with excellent particle size distribution. This behavior will support the goal of encapsulated extracted essential antimicrobial oils as a prospected target.

Table 2. The particle diameter of PLA and relative determined parameters.

Sample	Mean diameter, nm	Variance (PI)	Standard deviation	Chi Square
PLA	78.50	0.139	34.4 nm (43.9%)	41.410

3.5. Antimicrobial Activity of the tested Essential Oils

Contamination of foods processed by food borne pathogens is a major safety concern for food processors and consumers [33]. The preliminary screening of in vitro antibacterial activity of Cumin, Black seeds, Coriander, Black pepper, Lemongrass, Thyme, and Chamomile essential oils has been studied against *L. monocytogenes, E. coli O157: H7*, and *S. enteritidis* food borne pathogens using the disc diffusion technique. This method is useful for qualitatively comparing the antibacterial activities of various purified oil compounds and essential oils and against certain microorganisms.

The qualitative antibacterial activity of the essential oils shows a significant difference ($P < 0.05$) compared to the control treatment (Table 3). Lemongrass EO presented the largest inhibitory effect (30–35 mm) followed by Cumin EO (22–28 mm), then the Black seed EO (15–20 mm) and the least effective EO against the tested pathogens was Black pepper EO (8–10 mm). The density of antibacterial efficacy was in the following order: Lemongrass > Cumin > Black seed > Coriander > Thyme > Chamomile > Black pepper. *E. coli O157:H7* was the most resistant among the tested pathogens.

Table 3. Essential oils antimicrobial activity against food borne bacterial pathogens.

Essential oils	Inhibition zone diameter (mm) Bacterial food-borne pathogens		
	L. monocytogenes	*S. enteritidis*	*E. coli*
Control	$24.0^{a}_{b} \pm 0.0$	$22.0^{a}_{c} \pm 0.2$	$18.3^{b}_{c} \pm 0.2$
Cumin	$24.3^{b}_{b} \pm 0.6$	$28.7^{a}_{b} \pm 0.8$	$22.0^{b}_{b} \pm 0.2$
Black seed	$20.3^{a}_{c} \pm 0.4$	$18.0^{a}_{d} \pm 0.5$	$15.7^{b}_{d} \pm 0.6$
Coriander	$18.0^{a}_{c} \pm 0.2$	$16\ .3^{a}_{e} \pm 0.7$	$15.3^{b}_{d} \pm 0.3$
Black pepper	$10.0^{a}_{f} \pm 0.6$	$12.7^{a}_{f} \pm 0.4$	$8.2^{b}_{g} \pm 0.3$
Lemongrass	$35.0^{a}_{a} \pm 0.8$	$30.3^{b}_{a} \pm 0.5$	$25.0^{c}_{a} \pm 0.5$
Thyme	$16.0^{a}_{d} \pm 0.5$	$15.7^{a}_{e} \pm 0.0$	$11.0^{b}_{e} \pm 0.1$
Chamomile	$13.0^{a}_{e} \pm 0.0$	$12.7^{a}_{f} \pm 0.0$	$10.6^{b}_{e} \pm 0.5$

Note: Reference disc used was Fluclox 10 µg (control). The results are the mean ± SD (n = 3). Mean values followed by different superscript (within rows) and different subscripts (within columns) are significantly different ($P \leq 0.05$).

For further study, Lemongrass and Cumin EOs were selected on the basis of their highest inhibitory effect from inhibition zone test results. Among the tested extracts, the Lemongrass essential oil, revealed the largest antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), with (30–35 mm) inhibition zone [34]. Lemongrass EO was of the most broad-spectrum activity amongst the eleven essential oils evaluated for using as a food functional ingredient and showed satisfactory efficacy [35].

The essential oils biological activity depends on the chemical compositions of these oils which are affected by the environment and agronomic conditions. Several studies [35,37] have reported that the high content of citral (66%) substance, which is composed of geranial (29%) and isomeric neral (37%),

was responsible for the lemongrass EO activity. An inhibitory effect for Cumin EO against all tested bacterial strains was reported [38]. The essential oils main components are responsible for their antimicrobial activity, the antagonistic or synergistic action that occurs. Minor components may also contribute to the antimicrobial activity. Cumin high content of aldehyde (16.1%), â-pinene (11.4%), and limonene (3.1%), with their known antimicrobial effect, may be responsible for the antibacterial activity of Cumin EO. Also, Cumin EO minor components, geranyl acetate (1.7%), eugenol (0.7%), perillaldehyde (0.6%), R-pinene (0.6%), and sabinene (0.5%) are known bactericides and may contribute to antimicrobial activity [39].

3.6. Minimum inhibitory concentration (MIC)

Quantitative analysis of the Lemongrass, Cumin and their combination EOs was conducted to determine the best concentration that inhibits the growth of the three microorganisms under investigation (*S. enteritidis*, *E. coli O157:H7*, and *L. monocytogenes*). The inhibitory effect of elevated concentrations of essential oils against the tested strains is illustrated in Figure 3–5. Data showed that all screened EOs exhibited antibacterial activity in their different concentrations. In other words, MIC values for Lemongrass oil at 0.5% concentration can inhibit *L. monocytogenes* and *S. enteritidis* growth, while 1% inhibits *E. coli O157:H7*. With respect to MIC values for Cumin, 1% of the oil can inhibit the growth of *S. enteritidis*, 2% inhibit *L. monocytogenes* and *E. coli O157:H7*. Figure 3–5 show that mix oils (Lemongrass and Cumin) EOs have the highest activity against the tested strains where 0.5% of the mix oils is the lowest concentration that can inhibit the bacterial growth. The combination of Lemongrass with Cumin EOs resulted in synergistic positive interaction against tested bacteria. In combination tests, active compounds mix from both EOs, increased antibacterial activity. MICs are significantly lower for the mixture than those for a single EO. The pure EO activity was directly related to the action of volatiles on the microorganism, such as phenolic contents In addition, oils inhibition may be due to the presence of an aromatic ring with a polar functional group, and other factors, such as the hydrophilic/lipophilic balance and phenolic-OH groups, which are very reactive and can form hydrogen bonds easily with the active enzyme sites [40]. The results obtained confirmed the possible use of essential oils to protect against bacteria and extend the shelf-life in the food industry.

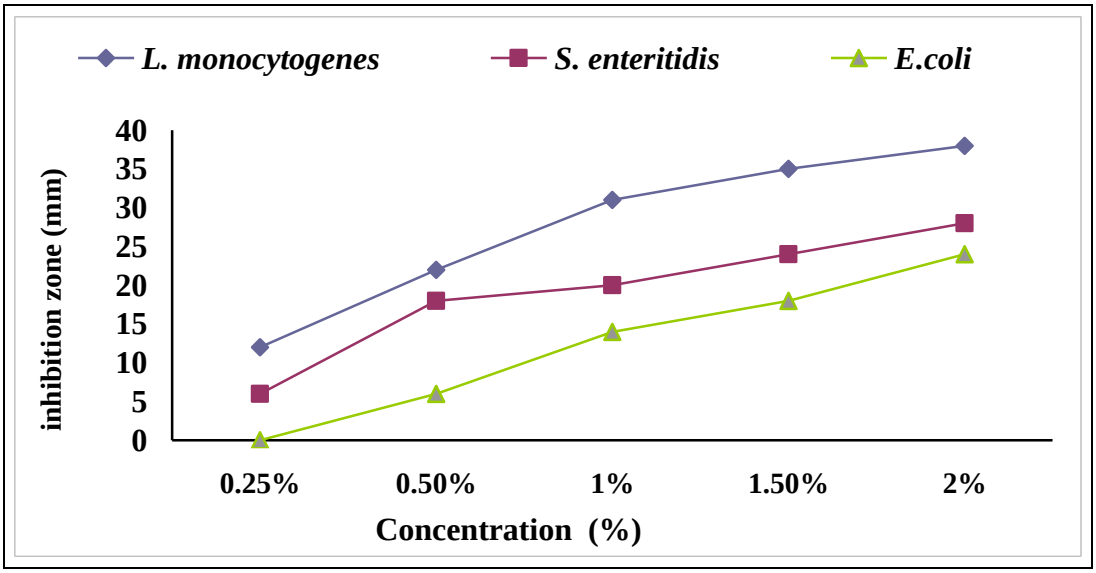


Figure 3. Minimum inhibitory concentration values of Lemongrass essential oil against tested bacterial strains.

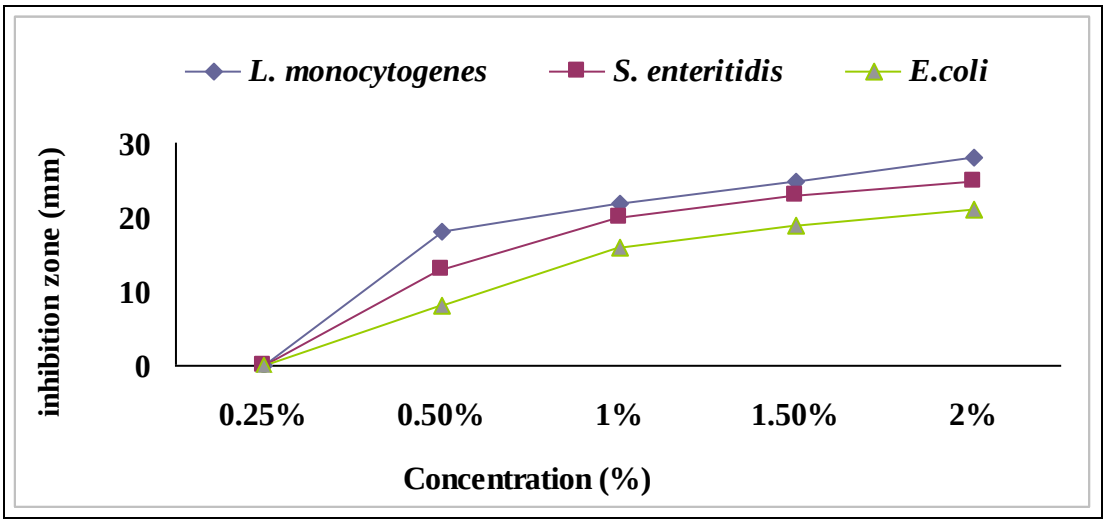


Figure 4. Minimum inhibitory concentration values of Cumin essential oil against tested bacterial strains.

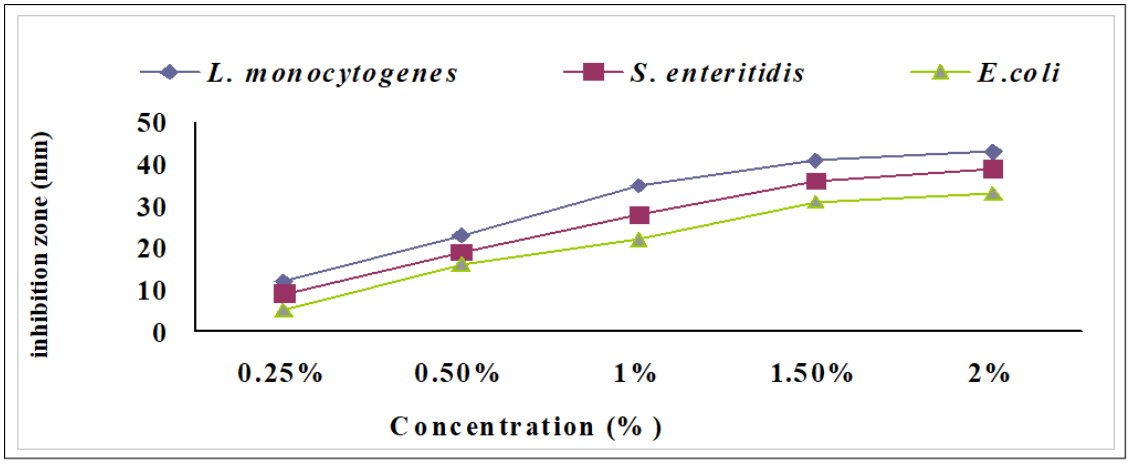


Figure 5. Minimum inhibitory concentration values of mix essential oils against tested bacterial strains.

3.7. Antibacterial effects of irradiated and non-irradiated coated PLA films with EOs

The results of the antimicrobial tests, presented in Table 4 (a) and (b), clearly demonstrate that the PLA film alone showed a considerable antibacterial action against the tested strains. PLA have a functional antimicrobial protection, and its use has been extended to the newly developed textile field [41]. In particular, they are especially suitable for items with single use, such as sanitary materials and specialized medical textiles. In contrast, pure PLA fiber and film did not exhibit any antibacterial activity [2,12].

Various ratios 0.25–2.0% (wt/vol) of Lemongrass, Cumin and their mix EOs were incorporated into PLA films and were tested against *S. enteritidis*, *E. coli O157:H7*, and *L. monocytogenes* for the area of inhibition zone Table 4 (a) and (b). The results showed a variation in the antibacterial activities of active films. The highest effect was achieved by the PLA/mix EOs, followed by PLA/Lemongrass EO (PLA/LGF) and PLA/Cumin EO (PLA/CF), respectively. The increase in the zone of inhibition against all the tested strains was proportional to the increase of essential oils concentrations. The inhibition zones against all of the tested bacteria reached their maximal values at oils concentration 2%.

Talebi et al., 2017 [2] reported a similar finding. They revealed a concentration-dependence for inhibitory activity of PLA films containing *Bunium persicum* and *Mentha piperita* essential oils. In another study, among the various formulations, the polylactide/polyethylene glycol/cinnamon oil film exhibited considerable antimicrobial activity against *S. typhimurium* and *L. monocytogenes* [6]. Oregano Essential Oil (OEO) addition into polylactic acid/polytrimenthylene carbonate (PLA/PTMC) blends significantly ($P < 0.05$), improved the antioxidant and antimicrobial capacities of the blends, The optimum balance between the mechanical, antioxidant, thermal and antimicrobial properties of the films was achieved by adding 9% by weight of OEO in PLA/PTMC blends [42]. Cinnamon essential oil (CEO) encapsulated in nanoparticles of chitosan (CS) and polylactic acid fibers have demonstrated high levels of inactivation toward *S. aureus* and *E. coli* [12].

Table 4 (a). Antibacterial effects of irradiated and un-irradiated coated PLA films with EOs.

Films	Inhibition zone diameter (mm) Microorganisms					
	Salmonella enteritidis			*Escherichia coli*		
	UI	5 kGy	10kGy	UI	5 kGy	10kGy
PE	NI	NI	NI	NI	NI	NI
PLAF[d]	$4.3^{a} \pm 0.05^{*}$	$4.8^{a} \pm 0.015$	$3.6^{b} \pm 0.05$	$2.0^{a} \pm 0.04$	$2.0^{a} \pm 0.01$	$1.0^{b} \pm 0.03$
PLA/ LGEOF[b]						
0.25%	$4.6^{a}{}_{d} \pm 0.04$	$4.2^{a}{}_{d} \pm 0.02$	$2.0^{b}{}_{d} \pm 0.01$	UI[c]	UI[c]	UI[c]
0.5%	$19.0^{a}{}_{c} \pm 0.03$	$18.3^{a}{}_{c} \pm 0.01$	$15.4^{b}{}_{c} \pm 0.04$	$13.5^{a}{}_{c} \pm 0.01$	$12.7^{a}{}_{c} \pm 0.02$	$10.0^{b}{}_{c} \pm 0.03$
1%	$35.3^{a}{}_{b} \pm 0.01$	$34.6^{a}{}_{b} \pm 0.02$	$30.0^{b}{}_{b} \pm 0.01$	$28.2^{a}{}_{b} \pm 0.03$	$28.6^{a}{}_{b} \pm 0.01$	$25.6^{b}{}_{b} \pm 0.02$
2%	$CI^{a}{}_{a} \pm 0.05$	$CI^{a}{}_{a} \pm 0.01$	$CI^{a}{}_{a} \pm 0.03$	$36.0^{a}{}_{a} \pm 0.01$	$35.2^{a}{}_{a} \pm 0.05$	$31.0^{b}{}_{a} \pm 0.01$
PLA/ CEOF[c]						
0.25	NI	NI	NI	NI	NI	NI
0.5%	$11.0^{a}{}_{c} \pm 0.03$	$10.6^{a}{}_{c} \pm 0.01$	$7.6^{b}{}_{c} \pm 0.05$	$7.0^{a}{}_{c} \pm 0.05$	$7.3^{a}{}_{c} \pm 0.02$	$4.3^{b}{}_{c} \pm 0.05$
1%	$26.7^{a}{}_{b} \pm 0.03$	$26.0^{a}{}_{b} \pm 0.04$	$21.7^{b}{}_{b} \pm 0.01$	$19.0^{a}{}_{b} \pm 0.05$	$18.6^{a}{}_{b} \pm 0.02$	$15.7^{b}{}_{b} \pm 0.01$
2%	$35.7^{a} \pm 0.05$	$35.3^{a} \pm 0.05$	$29.6^{b} \pm 0.05$	$26.3^{a}{}_{a} \pm 0.04$	$25.7^{a}{}_{a} \pm 0.01$	$21.6^{b}{}_{a} \pm 0.03$
PLA/MF[a]						
0.25%	$10.6^{a}{}_{d} \pm 0.02$	$10.0^{a}{}_{d} \pm 0.02$	$7.6^{b}{}_{d} \pm 0.03$	$4.7^{a}{}_{d} \pm 0.04$	$4.0^{a}{}_{d} \pm 0.00$	$2.7^{b}{}_{d} \pm 0.02$
0.5%	$36.2^{a}{}_{c} \pm 0.06$	$35.6^{a}{}_{c} \pm 0.01$	$32.2^{b}{}_{c} \pm 0.03$	$12.2^{a}{}_{c} \pm 0.03$	$11.7^{a}{}_{c} \pm 0.00$	$8.0^{b}{}_{c} \pm 0.01$
1%	$49.0^{a}{}_{b} \pm 0.07$	$48.7^{a}{}_{b} \pm 0.01$	$44.7^{b}{}_{b} \pm 0.00$	$36.4^{a}{}_{b} \pm 0.08$	$36.0^{a}{}_{b} \pm 0.01$	$30.6^{b}{}_{b} \pm 0.05$
2%	$CI^{a}{}_{a} \pm 0.06$	$CI^{a}{}_{a} \pm 0.02$	$CI^{a}{}_{a} \pm 0.01$	$42.3^{a}{}_{a} \pm 0.01$	$41.3^{a}{}_{a} \pm 0.01$	$37.0^{b}{}_{a} \pm 0.01$

Note: UI—Un-Irradiated; NI—No-Inhibition; CI—Complete Inhibition; PE—polyethylene; PLAF—PolyLactic Acid Film; PLA/LGEOF—PLA/Lemongrass essential oil film; PLA/CEOF—PLA/Cumin essential oil film; PLA/MF—PLA/mix Eos. *Mean values followed by different superscript (within rows) and different subscripts (within columns) are significantly different ($P \leq 0.05$). The results are the mean ± SD (n = 3).

In the current study, Lemongrass EO produced larger inhibition zone than Cumin EO when incorporated into PLA films (PLAF), with the same level of essential oil. Compared to PLA/CEOF, PLA/LGEOF demonstrated good stability and favorable sustained release of LGEO which resulted in improved antimicrobial activity.

Liakos et al., 2016 [43] recorded that antimicrobial activity of polylactic acid nanoparticles (NCs) has been enhanced due to the presence of lemongrass oil that has supported the development of well-separated NCs and has also improved antimicrobial properties due to the antibacterial properties of lemongrass. Lemongrass EO is composed of various molecules, including aldehydes, hydrocarbons, ketones, alcohols, etc. It can serve as a surfactant for PLA/LGEOF preparation due to the presence of long-chain molecules with both hydrophilic head and hydrophobic tail groups. In the presence of aldehydes (around 70% neral and geranial), Lemongrass EO can react with polylactic acid esters in an aldol reaction, resulting in stable PLA/LGEOF.

In this study, the lowest antibacterial effect was observed by PLA/CF, at concentration 0.25% this film was not effective against any tested strain, while the same concentration level of oils mix and lemongrass EOs showed inhibition zone against all tested strains. Generally, data revealed that the incorporation of mix EOs at 2% (v/v) shows a clear area due to the absence (complete inhibition) of *L. monocytogenes* and *S. enteritidis* growth around the film discs. At the same concentration the

clear inhibition zone was not observed with *E. coli*, which indicated that this bacterium was the most resistance among the tested bacteria.

Table 4 (b). Antibacterial effects of irradiated and un-irradiated coated PLA films with EOs.

Films	Inhibition zone diameter (mm) *Listeria monocytogenes*		
	UI	5 kGy	10 kGy
PE	NI	NI	NI
PLAF[d]	$16.0^{a} \pm 0.01$	$15.6.0^{a} \pm 0.00$	$14.6^{a} \pm 0.02$
PLA/ LGEOF[b]			
0.25%	$9.7^{a}{}_{d} \pm 0.04$	$10.0^{a}{}_{d} \pm 0.01$	$8.4^{b}{}_{d} \pm 0.00$
0.5%	$21.4^{a}{}_{c} \pm 0.07$	$20.6^{a}{}_{c} \pm 0.02$	$18.3.4^{b}{}_{c} \pm 0.04$
1%	$39.7^{a}{}_{b} \pm 0.04$	$40.0^{a}{}_{b} \pm 0.01$	$35.4^{b}{}_{b} \pm 0.00$
2%	$CI^{a}{}_{a} \pm 0.02$	$CI^{a}{}_{a} \pm 0.01$	$CI^{a}{}_{a} \pm 0.02$
PLA/ CEOF[c]			
0.25	NI	NI	NI
0.5%	$13.5^{a}{}_{c} \pm 0.08$	$12.7^{a}{}_{c} \pm 0.02$	$11.5^{b}{}_{c} \pm 0.03$
1%	$28.0^{a}{}_{b} \pm 0.02$	$26.0^{a}{}_{b} \pm 0.01$	$23.0^{b}{}_{b} \pm 0.03$
2%	$33.3^{a}{}_{a} \pm 0.033$	$32.0^{a}{}_{a} \pm 0.00$	$30.3^{b}{}_{a} \pm 0.013$
PLA/MF[a]			
0.25%	$11.4^{a}{}_{d} \pm 0.05$	$10.0^{a}{}_{d} \pm 0.00$	$9.3^{b}{}_{d} \pm 0.03$
0.5%	$35.0^{a}{}_{c} \pm 0.01$	$34.0^{a}{}_{c} \pm 0.02$	$31.0^{b}{}_{c} \pm 0.00$
1%	$43.0^{a}{}_{b} \pm 0.01$	$41.7^{a}{}_{b} \pm 0.05$	$39.3^{b}{}_{b} \pm 0.01$
2%	$CI^{a}{}_{a} \pm 0.04$	$CI^{a}{}_{a} \pm 0.03$	$CI^{a}{}_{a} \pm 0.01$

The results are the mean ± SD (n = 3). Mean values followed by different superscript (within rows) and different subscripts (within columns) are significantly different ($P \leq 0.05$).

From the foregoing results, it appeared that polylactic films incorporated with mix EO (PLA/MF) exhibited the highest inhibitory activity against the tested strains. Synergistic effects were reported for the combination of PLA films incorporated with *Ziziphora clinopodioides* EO (ZEO) and Ethanolic Propolis Extract (EPE) which showed significantly higher antibacterial effects against *S. aureus, L. monocytogenes*, *B. subtilis, B. cereus, S. enteritidi*s, and *E. coli O157:H7* than those obtained with ZEO or EPE alone ($P < 0.05$) [44]. In contrast PLA films containing *Bunium persicum* essential oil (BP EO) had significant antimicrobial activity ($P < 0.05$) more than *Mentha piperita* (MPEO) and their combination [2].

In general, two classes of antimicrobial agents may be used in the preparation of antimicrobial protection by polymer, which differs according to the antimicrobial activity mechanism. The first group includes antimicrobial agents that work through a controlled release mechanism. These agents are bound with physical forces to the polymer packaging, they can be released slowly into the surrounding area from the polymer or fiber in the presence of an appropriate amount of humidity where they prevent or completely destroy the growth of microorganisms. The second group includes agents operating on the basis of the principle of bio-barrier formation. In this instance, agents are chemically bound to the polymer packaging where the micro-organs which come into contact with polymer or fiber create a biological barrier [45,46]. In the current study, Incorporation of lemongrass

and cumin EOs in polylactic acid films (PLA/EOs), simultaneously produced dual antibacterial activity based on controlled release mechanisms and the formation of bio-barriers. Some researchers studied the effect of irradiation on the physical properties (structural, morphological, mechanical, and barrier properties) of packaging and packaging materials used in irradiated food packaging [47,48]. No information is available in the literature regarding the effect of irradiation on functional packaging (antimicrobial and antioxidant activity). This study examined the effect of γ irradiation at 5 and 10 kGy doses on PLA/LGEOF, PLA/CEOF and LPA/MF antibacterial activity, and data were reported (Table 4). All films exhibited zones of inhibition against selected bacterial pathogens.

The 5 kGy irradiation dose did not have any significant effect on PLA/LGEOF, PLA/CEOF, and PLA/MF antibacterial activity. The volatile content of non-irradiated and irradiated samples of the oils (responsible for antibacterial activity), was stable and did not degrade by irradiation. UV-A irradiation and accelerated Q-SUN had no effect on the antimicrobial activity of nano ZnO Methyl Hydroxypropyl Celluloses (MHPC) coatings against *B. cereus, S. aureus,* and *E. coli.* While Q-SUN irradiation reduced reduces the antibacterial effect of nanoparticle-containing MHPC coatings against *C. albicans* and *P. aeruginosa* [49].

Using γ-irradiation up to 5 kGy did not affect the antibacterial activity of active PLA/EOs significantly. The release of naphthalene from the polyamide-coated low-density polyethylene film differed with the irradiation dose, with the release rate by irradiation less than 0.25 kGy having no effect ($P > 0.05$) the dose range from 0.25 to 5.0 kGy reduced the release rates [48]. The cross-linking induced by radiation slightly influences the release rates at these dose ranges [49]. At these dosage levels, the crosslinking caused by ionizing radiation in the LDPE/polyamide polymer network of the LDPE/polyamide film resulted in the slow and gradual release of naphthalene [50]. Regardless of the different release rates between 0 and 5.0 kGy, the final cumulative amount of the released compound will reach the same level ($P > 0.05$) level (about 1.5 μg/mL). This result indicates that, at these doses, slow and progressive controlled release of active compounds (volatile oils) can be achieved by irradiation. However, in this study, when the irradiation dose was increased to 10 kGy, a significant decrease in the antibacterial activity of active PLA/EOs films was noticed. It can be concluded that during irradiation (10 kGy) a change in the composition of the active films speeds up the evaporation of the volatile oils. In contrast, Fourier Transform Infrared (FTIR) analysis of the structural conformation of cinnamaldehyde incorporated in polyamide-coated low-density polyethylene (LDPE) films revealed that, after ionizing radiation exposures up to a maximum of 10.0 kGy, its structural conformation was not affected. When the radiation dose increased to 20.0 kGy, changes in the trans-cinnamaldehyde functional group were induced [51]. The mechanism by which radiation causes changes in the composition of volatile oils incorporated in PLA film is not yet well known, but these changes may presumably be due to the sensitivity of the volatile oils components. The changes to the conformation of the molecules are due to irradiation. The changes also resulted from the oxidation and hydroxylation of terpenes aromatic rings and the potential degradation of certain constituents of essential oil during γ irradiation. This is in addition to the radiolytic effect and the potential production of free radicals [52].

Fourier Transform Infrared (FTIR) analysis of cinnamaldehyde structural conformation incorporated in polyamide-coated low-density polyethylene (LDPE) films showed that its structural conformation has not been affected after exposure to ionizing radiation up to 10.0 kGy [53].

4. Conclusions

Lemongrass and Cumin essential oils showed significant antimicrobial effects against *S. enteritidi*s, *E. coli O157:H7* and *L. monocytogenes.* The antibacterial activity of Lemongrass and Cumin essential oils and in combination between them (mixEOs) was maintained when incorporated into the PLA films. All active PLA/EOs films showed significant antimicrobial activity against the tested bacteria, PLA/mix EOs (PLAMF) exhibited the highest inhibitory activity against the tested bacteria, so it was selected for challenge tests (the future study). According to this study, exposure to γ irradiation dose (up to 5.0 kGy) leads to preserving the antibacterial activity of PLA/EOs films. The results are an initial step toward the development of self-sterile active packaging materials or functional packaging to be used in combination with the irradiation technique of food preservation.

Conflict of interest

The authors declare that there were no commercial or associative interests representing a conflict of interest in connection with this manuscript. Also, there were no financial and personal relationships with other people or organization, capable of inappropriately influence the report.

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