Nanomed Res J 5(3):225-233, Summer 2020

RESEARCH ARTICLE

In vitro antimicrobial effect of basil seed mucilage-chitosan films containing Ziziphora clinopodioides essential oil and MgO nanoparticles

Nikoo Naeeji, Yasser Shahbazi, Nassim Shavisi*

Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran

ARTICLE INFO

Article History:
Received 02 Jun 2020
Accepted 24 Jul 2020
Published 01 Aug 2020

Keywords:
Basil seed mucilage
Chitosan
Ziziphora clinopodioides
MgO nanoparticles

ABSTRACT

Objective(s): Edible films and coatings are becoming increasingly important in food preservation applications to maintain quality and extend shelf-life in perishable foods. The aim of this study was to investigate the effect of gamma irradiation at 0 and 5 KGy on in-vitro antimicrobial property of basil seed mucilage-chitosan films containing *Ziziphora clinopodioides* essential oil (ZEO) and MgO nanoparticles against *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus,* and *Bacillus subtilis*.

Methods: The gamma irradiation doses for fabricated films were 0 and 5 kGy using a 60Co source with a dose rate of 4.18 kGy/h at the Atomic Energy Organization of Iran (Tehran, Iran). The antimicrobial property of basil seed mucilage-chitosan films were evaluated using agar disk diffusion and broth micro-dilution assays.

Results: The main chemical composition of ZEO were found to be carvacrol (65.22%), thymol (19.51%), ɣ-terpinene (4.63%), and p-cymene (4.86%). The highest antimicrobial activity was found for film containing ZEO 2% + MgO nanoparticles 0.2% with inhibition zone and log DP of 5-7.9 mm and-1.76--3.33, respectively. S. *aureus* was the most sensitive bacteria for the prepared films with inhibition zone and log DP of 5.4-7.9 mm and-0.41--3.34, respectively. A smooth, compact, and homogeneous surface without grainy and porous structure was observed in the pure films. MgO nanoparticles and ZEO completely incorporated in the film matrices.

Conclusions: According to our results, it may be recommended that basil seed mucilage-chitosan films containing ZEO and MgO nanoparticles can be used for increasing shelf-life of stored food commodities.

How to cite this article
Naeeji N., Shahbazi Y., Shavisi N. In vitro antimicrobial effect of basil seed mucilage-chitosan films containing Ziziphora clinopodioides essential oil and MgO nanoparticles. Nanomed Res J, 2020; 5(3): 225-233. DOI: 10.22034/nmrj.2020.03.003

INTRODUCTION

Edible films and coatings are becoming increasingly important in food preservation applications to maintain quality and extend shelf-life in perishable foods [1]. Edible films keep safe from food products from mechanical damage, physical, chemical, and microbiological deteriorations [2]. Chitosan is a natural and non-toxic polysaccharide that is of much demand for both scientific studies and the food industry owing to its numerous applications including biodegradable films, blends, coatings, composites, and nanocomposites [3]. Compared to other edible film and coating compunds, chitosan has the advantage of being used together with functional substances such as minerals or vitamins, while it has the advantage of having antibacterial and antioxidant activity when used alone [4]. It also possess unique properties such as non-toxicity, abundance, biocompatibility, biodegradability, excellent film-forming ability, stability, and flexibility [5].

Basil (*Ocimum basilicum* L.) belongs of the

* Corresponding Author Email: *nassim.shavisi@yahoo.com*
nassimshavisi@razi.ac.ir

genus *Ocimum* and has been distributed in the tropical regions of Asia, Africa, and Central and South America [6]. Beside its traditional application as culinary herb and flavoring agent, the outer pericarp of seeds swells into a gelatinous mass upon wetting and can be used in combination with other film-forming materials [7]. The most important of functional properties of mucilage obtained from basil seeds including biodegradability, biocompatibility, heat-resistant, non-toxicity, low production cost, acceptability, and hydrophilic nature [8].

There is increasing interest about antimicrobial packaging films and coatings in the food industry. In this area, numerous studies have been focused in incorporating essential oils (EOs), bateriocins, nanofibers, and nanoparticles [9-12]. *Ziziphora clinopodioides* essential oil (ZEO) is defined by the U.S. Food and Drug Administration (U.S. FDA) as a generally considered as safe (GRAS) in food applications and is allowed to be used as a food additive or flavoring agent, as well as antimicrobial and antioxidant use [11]. Recent previous studies have indicated that ZEO has a approprite antibacterial activity in perishable food products [5,11,13,14]. Nano magnesium oxide (MgO) is also considered as a GRAS material by the U.S. FDA [15-17]. It has numerous advantages including excellent reproducibility, small particle size, large surface area, simplicity, and low cost [16].

Gamma irradiation is also recognized as a non-thermal preservative approach and has been extensivley applied for delaying of sprouting, the growth of pathogenic and spoilage microorganisms, disinfestations, and improvement of vulnerable foodstuffs shelf-life [18]. Previous studies indicated that gamma irradiation could improve antimicrobial activities of edible films/coatings containing antimicrobial compounds [19-21]. According to our knowledge, there were not any studies about the effects of gamma irradiation on *in vitro* antimicrobial property of basil seed mucilage-chitosan film. Moreover, there is a general lack of data for the *in vitro* antimicrobial property of basil seed mucilage-chitosan films supplemented with ZEO and MgO nanoparticles against the growth of food-borne pathogenic microorganisms. Our recent study [22] showed that the irradiated basil seed mucilage-chitosan films under the cobalt-60 source at 2.5 and 5 kGy doses had better tensile strength as well as lower swelling index and water vapor transmission rate compared with un-irradiated films. Moreover, rainbow trout fillets packaged with films enrichment with ZEO 2% + MgO 0.2% and ZEO 2% + MgO 0.1% had the lowest microbial property and chemical changes ($P < 0.05$) during prolonged refrigerated storage. Therefore, the aim of this study was to evaluate the effect of gamma irradiation at 0 and 5 KGy on *in-vitro* antimicrobial property of basil seed mucilage-chitosan films supplemented with ZEO and MgO nanoparticles against *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus,* and *Bacillus subtilis.*

MATERIALS AND METHODS

Materials

The whole part of fresh *Z. clinopodioides* was obtained from Gilan-e-Gharb city, Kermanshah province, west of Iran. The EO of *Z. clinopodioides* was acquired using hydro-distillation for 3.5 h using a Clevenger-type apparatus based on the method described in the European Pharmacopoeia [23]. The ZEO was collected, poured in an amber glass vial, and maintained at refrigerated temperature (4 ± 1 °C) until further use. The GC-MS analysis of the ZEO was conducted according to our previous study [24]. Chitosan was purchased from Sigma-Aldrich, Germany (deacetylation degree = 75-85% and a medium molecular weight = 450 KDa). MgO nanoparticles (diameter < 40 nm and purity > 99%) was obtained from the Iranian Nanomaterials Pioneers company (Razavi Khorasan, Iran). Basil seeds were obtained from a local market (Kermanshah, Iran) and identified in Razi University. All chemicals and culture media were obtained from Merck, Germany.

Extraction of basil seed mucilage

Isolation of basil seed mucilage was conducted based on the published method by Luo et al., (2019) [25] upon wetting (1:40 w/v, basil seed: water) at 50 ± 1 °C for 3 h. The obtained mucilage was dried and kept in a dry place until required for further experiments.

Preparation of basil seed mucilage-chitosan film

For preparation of basil seed mucilage-chitosan film, 2 g mucilage and 2 g chitosan individually dissolved in distilled water and acetic acid 1% for 3 and 8 h, respectively. Glycerol (0.75%, v/v of solution) was added into the solutions and mixed for 30 min. Films composed of 25 ml chitosan and 25 ml mucilage was prepared and then ZEO (1 and

2%) and MgO nanoparticles (0.1 and 0.2%) added. The film-forming solutions were casted in glass petri dishes and dried at 25 °C for 48 h [26].

Gamma irradiation of basil seed mucilage-chitosan films

The gamma irradiation doses for prepared films were 0 and 5 KGy using a ^{60}Co source with a dose rate of 4.18 kGy/h at the Atomic Energy Organization of Iran (Tehran, Iran).

Scanning electron microscopy of basil seed mucilage-chitosan films

Morphology of films and MgO nanoparticles was observed using a TeScan MIRA3 scanning electron microscope (SEM) using an accelerating voltage of 5 and 10 kV, respectively. The film samples (0.5 × 0.5 cm) were cut and prepared using dropping in liquid nitrogen. The prepared films were deposited on an aluminum holder and allowed to sputtering with gold/palladium alloy [13].

Bacterial strains

S. aureus (ATCC 6538), *L. monocytogenes* (ATCC 19118), *B. cereus* (ATCC 11774), and *B. subtilis* (ATCC 6633) were acquired from the microbial archive of Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran. Bacterial strains were cultured at 37 °C for 24 h in Brain Heart Infusion broth (BHI) medium and adjusted to a final density of 9 log CFU/ml.

In vitro antimicrobial activity of basil seed mucilage-chitosan films

The antimicrobial property of basil seed mucilage-chitosan films were examined using agar disk diffusion and broth micro-dilution methods.

For agar disk diffusion assay, 15 ml molted BHI agar was poured into petri dishes with a diameter of 90 mm. Then, 100 µl of the each test bacterial suspension, containing 9 log CFU/ml, was cultured on the BHI agar by surface method. The film pieces (6 mm in diameter) were put on the surface of inoculated BHI agar. After incubation at 37 ± 1 °C for 24 h, the diameter of the inhibition zones (mm) were determined [2].

For broth micro-dilution assay, the 96-well sterile micro-dilution plates with U-bottom wells were prepared by adding 180 µl of film forming solutions based on basil seed mucilage-chitosan containing ZEO (1 and 2%) and MgO nanoparticles (0.1 and 0.2%), and 20 µl of bacterial suspension containing 9 log CFU/ml of the test microorganisms. The positive (BHI broth containing 0.05 µl/ml of tetracycline and microorganism) and negative (BHI broth containing tested microorganisms) controls were also considered. The plates were sealed with sterile plate sealers. The micro-dilution plates were shacked on a plate shaker for 30 s and incubated at 37 ± 1 °C for 24 h. Afterward, sampling from each well was done using ten-fold serial dilutions with BHI broth, followed by plating on BHI agar and incubation for 24 h at 37 ± 1 °C. After incubation, the number of bacterial colonies was enumerated and the findings were exhibited in terms of differences in population (DP) according to the equation [27]:

$$\text{Log DP} = \log\left(\frac{N}{N^{\circ}}\right) = \log N - \log N_0$$

Where N and N_0 are the bacterial population (CFU/ml) at time t and zero, respectively.

Statistical analysis

All experiments were conducted in triplicate. The analysis was performed using SPSS 16.0 for Windows (SPSS, Chicago, IL, USA) software package. All results were subjected to one-way analysis of variance to determine the differences of samples. Significance level was considered $P < 0.05$ in all experimental data.

RESULTS AND DISCUSSION

Chemical composition of Ziziphora clinopodioides essential oil

As exhibited in our previous study [24], the main chemical composition of ZEO were determined to be carvacrol (65.22%), thymol (19.51%), *γ*-terpinene (4.63%), and *p*-cymene (4.86%). Ozturk and Ercisli, (2007) [28] indicated pulegone (31.8%), 1,8-cineole (12.2%) and limonene (10.4%) as the most corresponding compounds of ZEO. Aghajani et al., (2008) [29] indicated that *Z. clinopodioides* collected from Lorestan province contained 8.7% and 53.6% of carvacrol and thymol, respectively. In last years, Morteza-Semnani et al., (2005) [30] and Sonboli et al., (2010) [31] reported that terpineol, methyl acetate, and iso-neomenthol are the main composition of ZEO collected from various parts of Iran. In general, differences in chemical constituents of EOs obtained from medicinal herbs can be related to the herbal species, age, ecotypes, and other environmental factors [32].

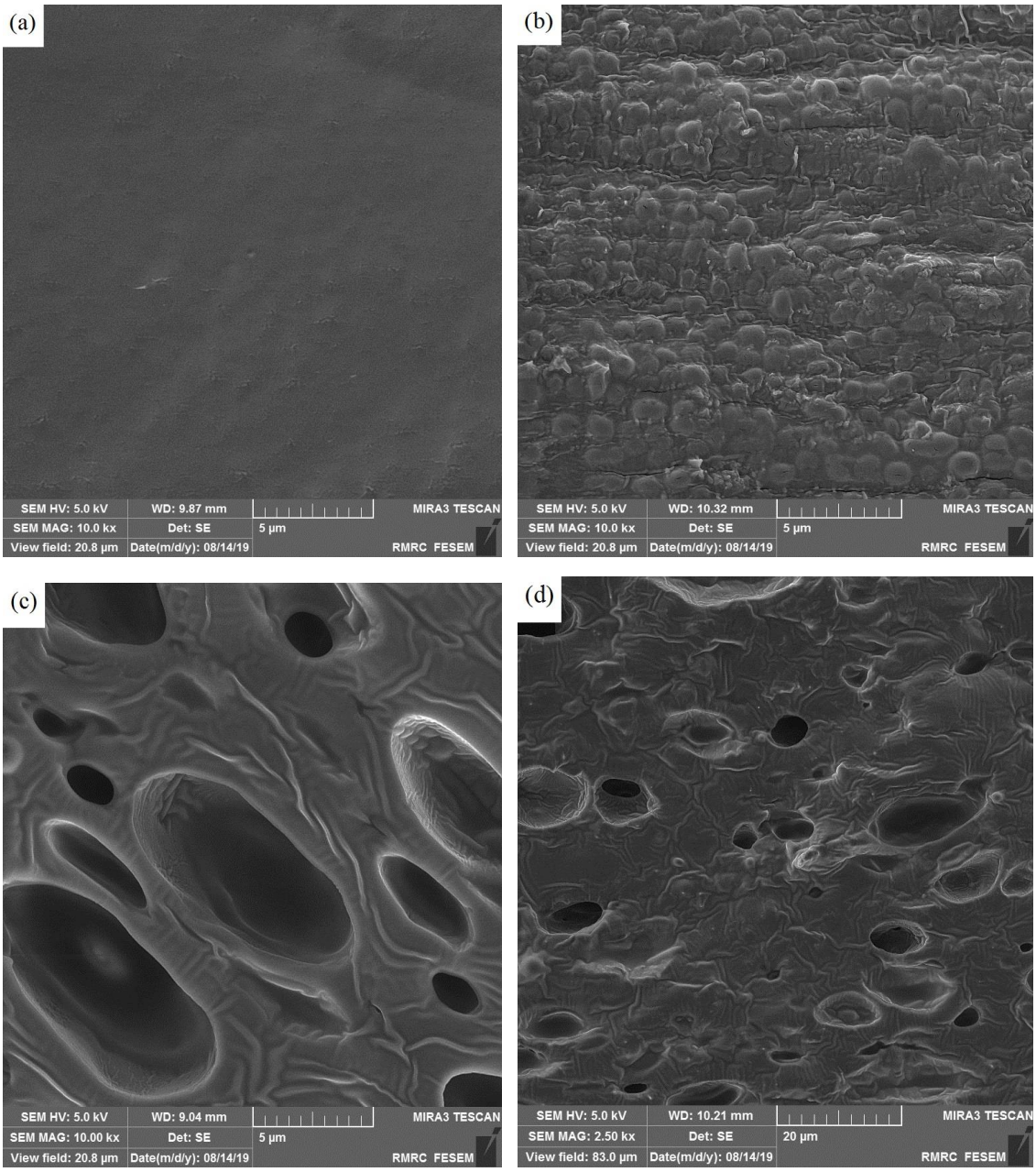

Fig. 1. Non-irradiated pure film (a), film + MgO 0.2% (b), film + ZEO 2% (c), and film + Mg 0.2% + ZEO 2% (d).

Scanning electron microscopy of basil seed mucilage-chitosan films

SEM images of non-irradiated (0 KGy) and irradiated films with 5 KGy are shown in Fig. 1a-d and 2a-d, respectively. A smooth, compact, and homogeneous surface without grainy and porous structure was observed in the pure films (Fig. 1a and 2a). Moreover, MgO nanoparticles completely incorporated in the film matrices (Fig. 1b and 2b). Sanuja et al., (2014) [33] also found that MgO nanoparticles completely dispersed in the chitosan film. Pores and cavities were found in the films containing ZEO (Fig. 1c-d and 2c-d), which may result from the volatility of the ZEO constituents [34,35]. These results are in general agreement with those reported for EO obtained from bergamot,

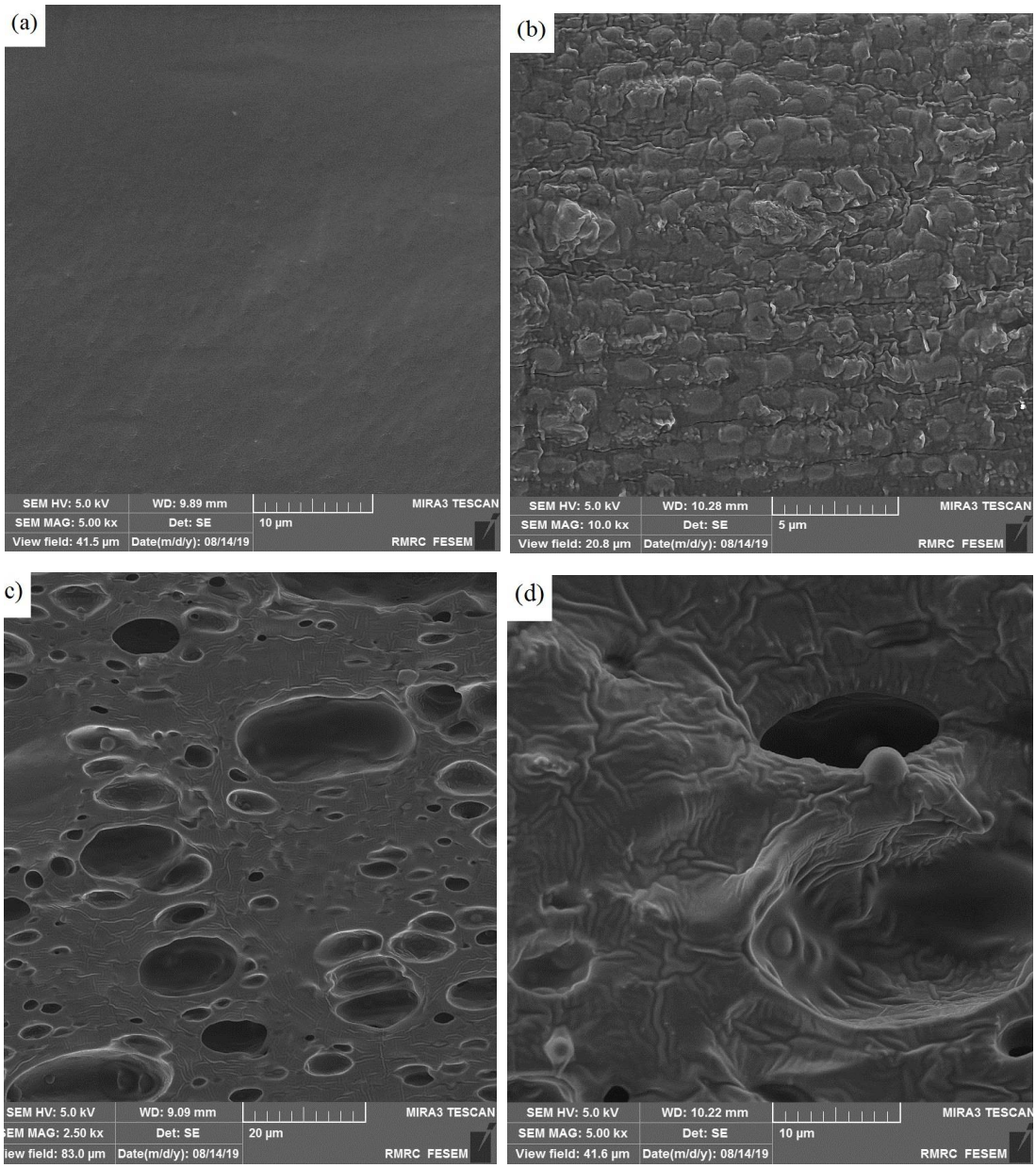

Fig. 2. Irradiated pure film (a), film + MgO 0.2% (b), film + ZEO 2% (c), and film + Mg 0.2% + ZEO 2% (d) with 5 KGy.

lemongrass [34], rosemary, cinnamon [36], thyme [37], and clove [33]. Moreover, based on SEM images, gamma irradiation with 5 KGy had not observed remarkable effects on the morphology of prepared films. Moreover, the SEM image of MgO nanoparticles are shown in Fig. 3.

In vitro antimicrobial activity of basil seed mucilage-chitosan films

The results of *in vitro* antimicrobial activity of basil seed mucilage-chitosan films are presented in Tables 1 and 2. Based on our findings presented in Table 1, for pure basil seed mucilage-chitosan film as a control group (without ZEO and MgO nanoparticles) and films containing MgO nanoparticles (0.1 and 0.2%) no inhibition zone was observed. After removing the films from agar plate, the pathogenic microorganisms did not growth in the agar plates. These results are in good agreement with those reported for chitosan film/coating with

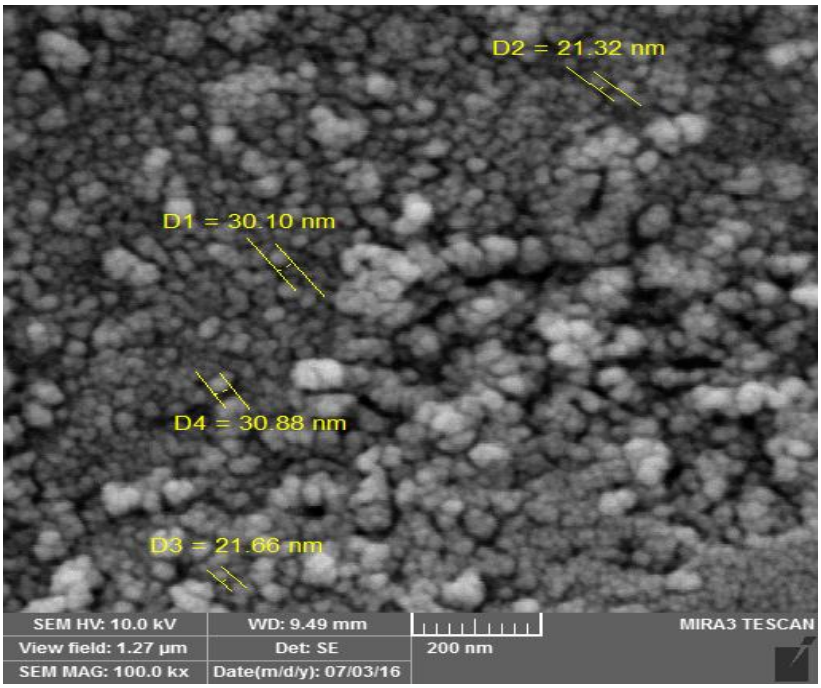

Fig. 3. SEM image of MgO nanoparticles.

Table 1. *In vitro* antibacterial activity (inhibition zone diameter; mm) of basil seed mucilage-chitosan films *Ziziphora clinopodioides* essential oil (ZEO) and MgO nanoparticles.

Film formulation	Dose (KGy)	*B. cereus*	*L. monocytogenes*	*B. subtilis*	*S. aureus*
Pure film	0	-	-	-	-
	5	-	-	-	-
Film + MgO 0.1%	0	-	-	-	-
	5	-	-	-	-
Film + MgO 0.2%	0	-	-	-	-
	5	-	-	-	-
Film + ZEO 1%	0	2.3 ± 0.1	4.2 ± 0.2	4.7 ± 0.2	5.5 ± 0.3
	5	2.3 ± 0.2	4.2 ± 0.1	4.7 ± 0.3	5.4 ± 0.3
Film + ZEO 2%	0	2.7 ± 0.1	6.2 ± 0.1	6.8 ± 0.4	7.0 ± 0.3
	5	2.7 ± 0.1	6.1 ± 0.3	6.8 ± 0.1	7.0 ± 0.1
Film + MgO 0.1% + ZEO 1%	0	3.1 ± 0.2	4.3 ± 0.2	5.3 ± 0.2	6.2 ± 0.2
	5	3.1 ± 0.2	4.3 ± 0.1	5.3 ± 0.1	6.2 ± 0.3
Film + MgO 0.2% + ZEO 1%	0	3.3 ± 0.1	4.5 ± 0.2	5.6 ± 0.2	6.7 ± 0.1
	5	3.4 ± 0.1	4.5 ± 0.1	5.6 ± 0.1	6.9 ± 0.1
Film + MgO 0.1% + ZEO 2%	0	4.5 ± 0.1	6.5 ± 0.2	7.2 ± 0.1	7.0 ± 0.1
	5	4.7 ± 0.3	6.8 ± 0.2	7.1 ± 0.1	7.3 ± 0.1
Film + MgO 0.2% + ZEO 2%	0	5.0 ± 0.1	7.2 ± 0.1	7.5 ± 0.1	7.9 ± 0.3
	5	5.1 ± 0.1	7.3 ± 0.3	7.5 ± 0.2	7.9 ± 0.1

inherent antimicrobial property [2,38]. Based on the results of broth-micro dilution assay (Table 2), the log DP of pure basil seed mucilage-chitosan film, film + MgO 0.1%, and film + MgO 0.2% were

Table 2. Log DP of basil seed mucilage-chitosan films *Ziziphora clinopodioides* essential oil (ZEO) and MgO nanoparticles.

Film formulation	Dose (KGy)	*B. cereus*	*L. monocytogenes*	*B. subtilis*	*S. aureus*
Pure film	0	-0.18 ± 0.01	-0.29 ± 0.02	-0.37 ± 0.01	-0.41 ± 0.03
	5	-0.19 ± 0.01	-0.30 ± 0.01	-0.35 ± 0.02	-0.41 ± 0.01
Film + MgO 0.1%	0	-0.35 ± 0.01	-0.56 ± 0.04	-0.66 ± 0.02	-0.89 ± 0.03
	5	-0.35 ± 0.05	-0.56 ± 0.07	-0.67 ± 0.03	-0.89 ± 0.02
Film + MgO 0.2%	0	-0.55 ± 0.02	-0.67 ± 0.03	-0.75 ± 0.01	-0.96 ± 0.02
	5	-0.54 ± 0.01	-0.69 ± 0.06	-0.75 ± 0.04	-0.97 ± 0.06
Film + ZEO 1%	0	-0.79 ± 0.05	-0.98 ± 0.08	-1.12 ± 0.09	-1.34 ± 0.23
	5	-0.79 ± 0.01	-0.97 ± 0.03	-1.13 ± 0.02	-1.34 ± 0.09
Film + ZEO 2%	0	-0.99 ± 0.04	-1.12 ± 0.01	-1.45 ± 0.03	-1.86 ± 0.07
	5	-0.99 ± 0.03	-1.23 ± 0.01	-1.45 ± 0.09	-1.86 ± 0.08
Film + MgO 0.1% + ZEO 1%	0	-1.54 ± 0.01	-1.87 ± 0.01	-2.12 ± 0.04	-2.24 ± 0.08
	5	-1.54 ± 0.04	-1.87 ± 0.01	-2.12 ± 0.01	-2.25 ± 0.02
Film + MgO 0.2% + ZEO 1%	0	-1.87 ± 0.05	-2.12 ± 0.19	-2.54 ± 0.27	-2.78 ± 0.06
	5	-1.87 ± 0.01	-2.12 ± 0.11	-2.56 ± 0.24	-2.76 ± 0.01
Film + MgO 0.1% + ZEO 2%	0	-1.32 ± 0.04	-2.56 ± 0.07	-2.76 ± 0.12	-2.80 ± 0.14
	5	-1.32 ± 0.05	-2.57 ± 0.04	-2.77 ± 0.01	-2.80 ± 0.03
Film + MgO 0.2% + ZEO 2%	0	-1.76 ± 0.02	-2.86 ± 0.07	-3.12 ± 0.08	-3.34 ± 0.13
	5	-1.77 ± 0.03	-2.86 ± 0.02	-3.12 ± 0.05	-3.33 ± 0.24

found to be -0.18 - -0.41, -0.35 - -0.89, and -0.54 - -0.97, respectively. Recent studies indicated that the antimicrobial property of chitosan is probably due to the interaction of its positively charged amino group with negatively charged bacterial cell membranes, which is cause the releasing of critical constituents of bacterial cell and kill them [12,39]. It has been suggested that reactive oxygen species released from MgO nanoparticles can attack to the bacteria such as *E. coli* O157:H7, *S. aureus*, and *Salmonella* Stanley and lead to cell death via disrupting the cell wall [15,40,41].

The inhibition zone and log DP of films containing ZEO 1% and 2% against bacteria were recorded to be 2.3-7 mm and -0.79 - -1.86, respectively (Tables 1 and 2). The *in vitro* antimicrobial activity of ZEO is also attributed to the main phenolic compounds which they release its proton increases the delocalization of double bonds and ATP in bacteria, also disrupt their cell wall and finally cell death [42]. The *in vitro* antibacterial activity of ZEO has been also conducted [24,28,43]. Behravan et al., (2007) [43] showed that the MICs of ZEO against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *S. Typhimurium*, and *Klebsiella pneumoniae* were 0.003, 0.033, 0.033, 0.067, and 0.067% (v/v), respectively. Ozturk & Ercisli, (2007) [28] indicated that ZEO had broad-spectrum antibacterial activity against *Bacillus* spp., *Moraxella catarrhali*, and *Enterobacter sakazakii* in broth micro-dilution assay. In this study, the highest antimicrobial activity was found for film containing ZEO 2% + MgO nanoparticles 0.2% with inhibition zone and log DP of 5-7.9 mm and -1.76 - -3.34, respectively. There are numerous pathway for combined effects of antimicrobial compounds such as suppressing the crucial enzymes, combinations of cell wall active agents, and application of cell wall active agents to increasing the receiving of other antimicrobials [44]. Previous studies also indicated that the antimicrobial properties of films varies depending on the properties of incorporated nanomaterials, molecular weight of polymers, preparation method, type of target microorganisms and solvents, and the diameter size of prepared films. It has been reported that the antibacterial activity of films with increasing size of diameters resulting in increased antibacterial property [45,46].

Our findings also showed that gamma irradiation of prepared films with 5 KGy has not effect on antimicrobial property of designated films. Zantar et al., (2015) [47] indicated that low dose of gamma irradiation (10 KGy) did not any effect on the antimicrobial activity of *Thymus vulgaris* and *Mentha pulegium* EOs, which is in agreement with our findings.

CONCLUSION

The main chemical composition of ZEO were found to be carvacrol (65.22%), thymol (19.51%), *γ*-terpinene (4.63%), and *p*-cymene (4.86%). According to our results, it may be recommended that basil seed mucilage-chitosan films containing ZEO and MgO nanoparticles can be used for increasing of shelf-life of stored food commodities.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

1. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. Food Chemistry. 2010;122(1):161-6.
2. Shahbazi Y. The properties of chitosan and gelatin films incorporated with ethanolic red grape seed extract and Ziziphora clinopodioides essential oil as biodegradable materials for active food packaging. International Journal of Biological Macromolecules. 2017;99:746-53.
3. Shahbazi Y. Application of carboxymethyl cellulose and chitosan coatings containing Mentha spicata essential oil in fresh strawberries. International Journal of Biological Macromolecules. 2018;112:264-72.
4. Duan J, Jiang Y, Cherian G, Zhao Y. Effect of combined chitosan-krill oil coating and modified atmosphere packaging on the storability of cold-stored lingcod (Ophiodon elongates) fillets. Food Chemistry. 2010;122(4):1035-42.
5. Mohebi E, Shahbazi Y. Application of chitosan and gelatin based active packaging films for peeled shrimp preservation: A novel functional wrapping design. LWT - Food Science and Technology. 2017;76:108-16.
6. Razavi SMA, Mortazavi SA, Matia-Merino L, Hosseini-Parvar SH, Motamedzadegan A, Khanipour E. Optimisation study of gum extraction from Basil seeds (Ocimum basilicumL.). International Journal of Food Science & Technology. 2009;44(9):1755-62.
7. Hashemi SMB, Mousavi Khaneghah A. Characterization of novel basil-seed gum active edible films and coatings containing oregano essential oil. Progress in Organic Coatings. 2017;110:35-41.
8. Hashemi Gahruie H, Ziaee E, Eskandari MH, Hosseini SMH. Characterization of basil seed gum-based edible films incorporated with Zataria multiflora essential oil nanoemulsion. Carbohydrate Polymers. 2017;166:93-103.
9. Vejdan A, Ojagh SM, Adeli A, Abdollahi M. Effect of TiO2 nanoparticles on the physico-mechanical and ultraviolet light barrier properties of fish gelatin/agar bilayer film. LWT - Food Science and Technology. 2016;71:88-95.
10. Wang H, Gong X, Miao Y, Guo X, Liu C, Fan Y-Y, et al. Preparation and characterization of multilayer films composed of chitosan, sodium alginate and carboxymethyl chitosan-ZnO nanoparticles. Food Chemistry. 2019;283:397-403.
11. Rezaei F, Shahbazi Y. Shelf-life extension and quality attributes of sauced silver carp fillet: A comparison among direct addition, edible coating and biodegradable film. LWT. 2018;87:122-33.
12. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry. 2010;120(1):193-8.
13. Khezrian A, Shahbazi Y. Application of nanocompostie chitosan and carboxymethyl cellulose films containing natural preservative compounds in minced camel's meat. International Journal of Biological Macromolecules. 2018;106:1146-58.
14. Shavisi N, Khanjari A, Basti AA, Misaghi A, Shahbazi Y. Effect of PLA films containing propolis ethanolic extract, cellulose nanoparticle and Ziziphora clinopodioides essential oil on chemical, microbial and sensory properties of minced beef. Meat Science. 2017;124:95-104.
15. Sanuja S, Agalya A, Umapathy MJ. Studies on Magnesium Oxide Reinforced Chitosan Bionanocomposite Incorporated with Clove Oil for Active Food Packaging Application. International Journal of Polymeric Materials and Polymeric Biomaterials. 2014;63(14):733-40.
16. Jin T, He Y. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. Journal of Nanoparticle Research. 2011;13(12):6877-85.
17. De Silva RT, Mantilaka MMMGPG, Ratnayake SP, Amaratunga GAJ, de Silva KMN. Nano-MgO reinforced chitosan nanocomposites for high performance packaging applications with improved mechanical, thermal and barrier properties. Carbohydrate Polymers. 2017;157:739-47.
18. Jay JM, Loessner MJ, Golden DA. Modern food microbiology, 7th ed.,. New York, NY: Springer Science Business Media, Inc,; 2005.
19. Akhavan A, Khoylou F, Ataeivarjovi E. Preparation and characterization of gamma irradiated Starch/PVA/ZnO nanocomposite films. Radiation Physics and Chemistry. 2017;138:49-53.
20. Hamdani AM, Wani IA, Bhat NA. Effect of gamma irradiation on the physicochemical and structural properties of plant seed gums. International Journal of Biological Macromolecules. 2018;106:507-15.
21. Madera-Santana TJ, Meléndrez R, González-García G, Quintana-Owen P, Pillai SD. Effect of gamma irradiation on physicochemical properties of commercial poly(lactic acid) clamshell for food packaging. Radiation Physics and Chemistry. 2016;123:6-13.
22. Naeeji N, Shahbazi Y, Shavisi N. Effect of gamma irradiation on physico-mechanical and structural properties of basil seed mucilage-chitosan films containing Ziziphora clinopodioides essential oil and MgO nanoparticles for rainbow trout packaging. Journal of Food Processing and Preservation. 2020.
23. European pharmacopoeia. 3th ed. Royal society of medicine press, strasbourg 1997. 21-7 p.
24. Shahbazi Y, Shavisi N. Interactions of Ziziphora clinopodioides and Mentha spicata essential oils with chitosan and ciprofloxacin against common food-related pathogens. LWT - Food Science and Technology. 2016;71:364-9.
25. Luo M, Cao Y, Wang W, Chen X, Cai J, Wang L, et al. Sustained-release antimicrobial gelatin film: Effect of chia mucilage on physicochemical and antimicrobial properties. Food Hydrocolloids. 2019;87:783-91.
26. Siripatrawan U, Harte BR. Physical properties and antioxidant

activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloids. 2010;24(8):770-5.
27. Pérez-Rodríguez F, Posada-Izquierdo GD, Valero A, García-Gimeno RM, Zurera G. Modelling survival kinetics of Staphylococcus aureus and Escherichia coli O157:H7 on stainless steel surfaces soiled with different substrates under static conditions of temperature and relative humidity. Food Microbiology. 2013;33(2):197-204.
28. Ozturk S, Ercisli S. Antibacterial activity and chemical constitutions of Ziziphora clinopodioides. Food Control. 2007;18(5):535-40.
29. Aghajani Z, Assadian F, Masoudi S, Chalabian F, Esmaeili A, Tabatabaei-Anaraki M, et al. Chemical composition and in vitro antibacterial activities of the oil of Ziziphora clinopodioides and Z. capitata subsp. capitata from Iran. Chemistry of Natural Compounds. 2008;44(3):387-9.
30. Morteza-Semnani K, Saeedi M, Eslami G. Essential Oil Composition ofZiziphora clinopodioidesLam. From Iran. Journal of Essential Oil Bearing Plants. 2005;8(2):208-12.
31. Sonboli A, Atri M, Shafiei S. Intraspecific Variability of the Essential Oil of Ziziphora clinopodioides ssp. rigida from Iran. Chemistry & Biodiversity. 2010;7(7):1784-9.
32. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology. 2004;94(3):223-53.
33. Sanuja S, Agalya A, Umapathy MJ. Studies on Magnesium Oxide Reinforced Chitosan Bionanocomposite Incorporated with Clove Oil for Active Food Packaging Application. International Journal of Polymeric Materials and Polymeric Biomaterials. 2014;63(14):733-40.
34. Ahmad M, Benjakul S, Prodpran T, Agustini TW. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. Food Hydrocolloids. 2012;28(1):189-99.
35. Ghasemlou M, Aliheidari N, Fahmi R, Shojaee-Aliabadi S, Keshavarz B, Cran MJ, et al. Physical, mechanical and barrier properties of corn starch films incorporated with plant essential oils. Carbohydrate Polymers. 2013;98(1):1117-26.
36. Dong Z, Xu F, Ahmed I, Li Z, Lin H. Characterization and preservation performance of active polyethylene films containing rosemary and cinnamon essential oils for Pacific white shrimp packaging. Food Control. 2018;92:37-46.
37. Shojaee-Aliabadi S, Mohammadifar MA, Hosseini H, Mohammadi A, Ghasemlou M, Hosseini SM, et al. Characterization of nanobiocomposite kappa-carrageenan film with Zataria multiflora essential oil and nanoclay. International Journal of Biological Macromolecules. 2014;69:282-9.
38. Nowzari F, Shábanpour B, Ojagh SM. Comparison of chitosan–gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout. Food Chemistry. 2013;141(3):1667-72.
39. Aşik E, Candoğan K. Effects of Chitosan Coatings Incorporated with Garlic Oil on Quality Characteristics of Shrimp. Journal of Food Quality. 2014;37(4):237-46.
40. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. Journal of Microbiological Methods. 2003;54(2):177-82.
41. Sawai J, Kojima H, Igarashi H, Hashimoto A, Shoji S, Sawaki T, et al. Antibacterial characteristics of magnesium oxide powder. *World Journal of Microbiology and Biotechnology* 2000;16(2):187-94.
42. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control. 2014;46:412-29.
43. Behravan J, Ramezani M, Hassanzadeh MK, Eskandari M, Kasaian J, Sabeti Z. Composition, Antimycotic and Antibacterial Activity ofZiziphora clinopodioidesLam. Essential Oil from Iran. Journal of Essential Oil Bearing Plants. 2007;10(4):339-45.
44. Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Research International. 2011;44(9):3057-64.
45. Szunerits S, Boukherroub R. Antibacterial activity of graphene-based materials. Journal of Materials Chemistry B. 2016;4(43):6892-912.
46. Aider M. Chitosan application for active bio-based films production and potential in the food industry: Review. LWT - Food Science and Technology. 2010;43(6):837-42.
47. Zantar S, Haouzi R, Chabbi M, Laglaoui A, Mouhib M, Mohammed B, et al. Effect of gamma irradiation on chemical composition, antimicrobial and antioxidant activities of Thymus vulgaris and Mentha pulegium essential oils. Radiation Physics and Chemistry. 2015;115:6-11.