Journal of Human Environment and Health Promotion

Print ISSN: 2476-5481 Online ISSN: 2476-549X

The Inhibition of Escherichia coli O157:H7 Inoculated in Hamburger Using a Chitosan/Cellulose Nanofiber Film Containing the Nanoemulsion of Trachyspermum ammi and Bunium persicum Essential Oils

Batool Soltaninezhad [a] | **Saeid Khanzadi** [a *] | **Mohammad Hashemi** [b,c] | **Mohammad Azizzadeh** [d]

a. Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
b. Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
c. Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
d. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

***Corresponding author:** Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. Postal code: 9177948974.
E-mail address: Khanzadi@um.ac.ir

ARTICLE INFO

Article type:
Original article

Article history:
Received: 12 November 2019
Revised: 22 December 2019
Accepted: 6 January 2020

DOI: 10.29252/jhehp.6.1.6

Keywords:
Trachyspermum ammi
Bunium persicum
Chitosan
Escherichia coli O_{157}:H_7
Nanoemulsion

ABSTRACT

Background: Antimicrobial compounds have numerous applications, and essential oils could be used in edible films to enhance the food shelf life. This study aimed to assess the inhibitory effects of a chitosan/cellulose nanofiber (CNF) film containing the nanoemulsions of *Bunium persicum* essential oil (NBPEO) and *Trachyspermum ammi* essential oil (NTEO) on *Escherichia coli* O_{157}:H_7 inoculated in hamburger during storage (4°C).

Methods: After inoculation, the hamburger samples were classified into three treatment groups of, control, chitosan containing 7.5% CNF (Ch-CNF), and chitosan containing 7.5% CNF enriched with 1.6% NTEO and 0.8% NBPEO (Ch-CNF-NEO). The samples were preserved at the temperature of 4°C, and the bacterial counts were determined on days zero, three, six, nine, and 12. Data analysis was performed using Bonferroni post-hoc test and repeated measures ANOVA.

Results: The mean *E.coli* O_{157}:H_7 count significantly decreased in the treatments groups compared to the control group. In addition the Ch-CNF-NEO film exerted the most significant inhibitory effects on the growth of *E. coli* O_{157}: H_7 in the hamburger samples.

Conclusion: According to the results, the Ch-CNF-NEO film could effectively reduce the growth of *E. coli* O_{157}:H_7 in hamburger. Therefore, Ch-CNF-NEO film could be used to effectively increase the safety of meat products against *E. coli* O_{157}: H_7.

1. Introduction

Meat is a perishable food and requires proper maintenance. When stored at the temperature of 4 ºC, the shelf life of fresh beef is extremely low (3-5 days), which is attributed to the high water content and chemical composition of the food. These factors often facilitate hazardous microbial growth, thereby leading to the spoilage of meat [1]. Although preventive measures are observed in animal slaughter and proper hygiene and productive measures are also taken in the other stages of meat processing, pathogenic bacterial contamination remains an important health threat in the case of raw meat and other meat products. Some of the most harmful pathogens in this regard include *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., and pathogenic verotoxin-producing *E. coli* [2]. The outbreaks of foodborne diseases have been reported in various food products, while foods of bovine

How to cite: Soltaninezhad B, Khanzadi S, Hashemi M, Azizzadeh M. The Inhibition of *Escherichia coli* O_{157}:H_7 Inoculated in Hamburger Using a Chitosan/ Cellulose Nanofiber Film Containing the Nanoemulsion of *Trachyspermum ammi* and *Bunium persicum* Essential Oil. *J Hum Environ Health Promot.* 2020; 6(1): 30-4.

origin are considered to be the main carriers of *E. coli* O_{157}:H_7 infections in humans [3]. The Shiga toxigenic *E. coli* O_{157}:H_7 outbreaks have been previously reported in hamburger [4].

A novel approach to enhancing the shelf life of food products and their preservation involves the use of active, edible films, which mainly contain antimicrobial agents [1]. Various biopolymers are used in the production of such film. Such example is, Chitosan, which is extensively used in the production of films and gels to reinforce the organoleptic properties of foods, such as oxygen/humidity permeability and functional substances (e.g., flavoring agents, vitamins, coloring agents, and antioxidants) [5]. In general, antimicrobial agents are combined with polymeric materials (e.g., chitosan) in order to produce effective antimicrobial films [6].

Cellulose is the most abundant polysaccharide in nature. Recently, cellulose nanofiber (CNF) has been employed in the production of nanocomposite polymers. CNF has remarkable potential in the reinforcement of nanocomposites. Among the other prominent features of CNF are the low density, renewable nature, high strength, and modulus. In addition, CNF could improve some of the physical, mechanical, optical, and thermal properties of film components [7].

Essential oils (EOs) are extracted from the flowers, buds, seeds, leaves, bark, herbs, fruits, and roots of various plants [8]. EOs are secondary metabolites that, could inhibit the growth of the microbial pathogens that cause various foodborne diseases and food spoilage [5]. *Bunium persicum* belongs to the *Apiaceae* family and grows in regions such as the central Asia, Iran, Pakistan, Afghanistan, and India [9]. *Bunium persicum* EO is an abundant source of antibacterial agents owing to the high concentrations of oxygenated monoterpenes, including γ-terpinene, cuminaldehyde, ρ-cymene, and limonene [5]. *Trachyspermum ammi* is another member of the *Apiaceae* family, which has analgesic, anti-inflammatory, anxiolytic, and antispasmodic properties [10]. *Trachyspermum ammi* is an aromatic seed, which is mainly used as a spice and originates, in the Middle East, India, Iran, Afghanistan, and Egypt. The EO content of the ripe seeds of the plant has been estimated at 2-4% [11].

Nanotechnology has recently attracted the attention of industrialists and scientists for food production [12]. Nanoemulsion is defined as the dispersion of oil in water, with the droplet diameter of 10-100 nanometers [13]. According to the literature, EO nanoemulsions are able to reinforce the bioactive effects of EOs, thereby decreasing the applied concentration of EOs and the effects on the taste and aroma of food [14,15]. To date, no studies have been focused on the effects of films containing *Bunium persicum* essential oil (NBPEO) and *Trachyspermum ammi* essential oil (NTEO) on the inhibition of food pathogens in hamburger. The present study aimed to evaluate the effects of the chitosan/CNF film containing the nanoemulsions of NBPEO and NTEO on the growth of *E. coli* O_{157}:H_7 inoculated in hamburger in storage conditions at the temperature of 4 ± 1°C.

2. Materials and Methods

2.1. Experimental Materials

Chitosan powder was purchased from Sigma-Aldrich (St. Louis, MO, USA) with the medium molecular weight of 450 kDa, and CNF was provided by Nano Novin Polymer (Mazandaran, Iran). The EOs were provided by Nader Mashhad Industry and Cultivate Company (Mashhad, Iran). The applied culture media included the brain heart infusion (BHI) broth, BHI agar and Sorbitol-MacConkey agar which, were purchased from Quelab (Quelab Laboratories Inc., Montreal, Canada). *E. coli* O_{157}:H_7 (NCTC 12900) was obtained from the culture collection of the Department of Food Hygiene at the School of Veterinary Sciences of Ferdowsi University of Mashhad, Iran.

2.2. Preparation of the Nanoemulsions

The EO nanoemulsions were prepared based on the method proposed by Hashtjin *et al.* (2015) with modifications. The oil-in-water nanoemulsions were prepared using the NTEO and NBPEO (2% w/w) as the oil phase and, Tween 80 (2% w/w), and deionized water (96% w/w) as the aqueous phase (2% NTEO and NBPEO with 2% Tween 80). The emulsions were prepared in two stages. Initially, the oil and aqueous phases (total: 100 g) were placed in a glass beaker and mixed for 15 minutes at room temperature (25°C) using a magnetic stirrer (700 rpm). Afterwards, the obtained nanoemulsions were sonicated (SONOPULS Ultrasonic Homogenizers; BANDELIN, Berlin, Germany) at 50% amplitude for 15 minutes (pulse: 45 s, rest: 15 s). During the sonication process, the glass beaker was placed in an ice container in order to control the temperature [16]. Following that, the particle size of the nanoemulsions was measured using a dynamic light scattering (DLS) device (Nanophox Sympatec GmbH, Clausthal, Germany).

2.3. Preparation of the Chitosan /CNF Film

To prepare the chitosan solution (2% w/v in 1% v/v acetic acid), two grams of chitosan was mixed with 100 milliliters of distilled water. Afterwards, the obtained solution was stirred for one hour using a hotplate magnetic stirrer in order to achieve transparency. At the next stage, glycerol was selected as the plasticizer, added to chitosan (0.75 ml/g), and mixed for 30 minutes. Simultaneously, 7.5% CNF was added to the solution, and stirring continued for another 30 minutes. Following that, the chitosan solutions were placed at the center of teflon plates and dried at an ambient temperature (25°C) for 48 hours [17].

2.4. Preparation of the Chitosan/CNF/NTEO/NBPEO Film

In order to prepare the film containing NBPEO and NTEO, the EO nanoemulsions were added to the chitosan solution. After achieving the final NBPEO concentration of 0.8% and NTEO concentration of 1.6% (v/v), the film-forming solutions were placed at the center of teflon plates and dried at an ambient temperature (25°C) for 48 hours [17].

2.5. Preparation of E. coli O_{157}:H_7

At this stage, E. coli O_{157}:H_7 was cultured on the BHI broth and incubated at the temperature of 37 °C for 24 hours. Following that, the cultured samples were re-incubated at the temperature of 37 °C for another 18 hours. The 18- hour culture was also performed to obtain the bacterial suspension with 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml) [18].

2.6. Preparation of the Treatments

After purchasing beef and fat from a local market, a three-millimeter plate was used to grind the meat samples. Based on the weight of the meat, hamburgers were prepared by mixing the ground lean beef with beef fat (12.5%), potable water (10%), salt (1%), onion powder (1%), garlic powder (1%), and black pepper (0.5%). Following that, the bacterial suspension was used for the direct inoculation of the hamburgers with the final bacterial concentration of ~10^6 CFU/g [19]. To prepare the hamburgers, 50 grams of the mixture was placed on a plastic wrap and pressed by a manual hamburger press machine. The mean diameter and thickness of the hamburgers was 11 and 0.8 centimeters, respectively. Both surfaces of the hamburgers were coated with the film formulations, while the control samples had no coating (Table 1). At the next stage, the prepared samples were stored at the temperature of 4 ± 1 °C for 12 days, and periodic microbiological analysis was performed on days zero, three, six, nine, and 12.

2.7. Microbial Analysis

The hamburgers (10 g) were brought to the final volume of 90 milliliters of 0.1% sterile peptone water and homogenized for three minutes using a stomacher (Seward Medical, London, UK). After the preparation of the decimal dilutions, the drop plate method was employed at 10 microliters in order to culture the homogenate serial dilutions onto the Sorbitol-MacConkey agar, and the homogenates were incubated at the temperature of 37 °C for 24 hours. Finally, the bacterial counts were expressed as $\log_{10}$ CFU/g of the samples [18].

2.8. Statistical Analysis

Data analysis was performed in SPSS version 21 (SPSS Inc., Chicago, USA) using one-way analysis of variance (ANOVA), Bonferroni post-hoc test, and Dunnett test at the significance level of $P < 0.05$. All the experiments were performed in triplicate.

3. Results and Discussion

The particle size of NTEO and NBPEO was measured using a dynamic light scattering (DLS) device, and estimated at 90.21 and 97 nanometers, respectively. The optimal size of the nanoparticles was within the range of 10-100 nanometers [13].

Initially, the *E. coli* O_{157}:H_7 count was estimated at 5.5 log CFU/g in the control group, which was higher compared to the treatment groups (Figure 1). However, the value decreased to 5.3 log CFU/g after the storage period. The final bacterial counts in the treatment groups were estimated at 4.5 and 4 log CFU/g with the Ch-NCF and Ch-CNF-NEO films, respectively.

Table 1: List of treatments

No	Treatment	Description
1	CON	Control: Hamburgers without any film
2	Ch-CNF	Hamburgers with chitosan film containing 7.5% cellulose nanofiber without any nanoemulsion of essential oil
3	Ch-CNF-NEOs	Hamburgers with chitosan film containing 7.5% cellulose nanofiber, 0.8% *Bunium persicum* essential oil and 1.6% *Trachyspermum ammi* essential oil

According to the findings of the current research, the growth of *E. coli* O_{157}:H_7 reduced at the temperature of 4 ± 1°C in the treatment groups, which is in line with the previous studies in this regard [5, 18]. Furthermore, the Ch-NCF and Ch-CNF-NEO films had significant differences with the non-coated hamburger samples ($P < 0.05$), which is consistent with the previous findings in this regard [5, 20, 21]. Contrary to these results, Shekarforoush *et al.* (2015) reported that chitosan had no inhibitory effects against *E. coli* O_{157}:H_7 inoculated in ready-to-eat chicken meat stored at various temperatures (3°C, 8°C, and 20 °C) [22]. Chitosan activity is largely affected by incubation temperature, bacterial strain, and type of food substrate [21]. In the present study, the inhibitory effect of the Ch-CNF-NEO film on *E. coli* O_{157}:H_7 was more significant compared to the Ch-NCF film ($P < 0.05$). In a similar research, Zivanovic *et al.* (2005) used pure chitosan films, reporting the reduction of the *E. coli* O_{157}:H_7 count in bologna. In the mentioned study, the chitosan films were enriched with 1% and 2% oregano EO and proved adequate for the reduction of *E. coli* O_{157}:H_7 by three logs on the bologna slices [20]. In another research, Raji *et al.* (2019) used the combination treatment of chitosan coating and, EOs (*Zataria multiflora* and *Bunium persicum*) in rainbow trout fillets, and the application of vacuum packs was reported to diminish the *E. coli* count to undetectable levels [5]. Several studies have confirmed the antibacterial activity of chitosan films enriched with various EOs against *E. coli* O_{157}:H_7 [6, 20]. For instance, Shekarforoush *et al.* (2015) claimed that the combination of chitosan and oregano EO had antibacterial activity against *E. coli* O_{157}:H_7 in ready-to-eat chicken [22]. Furthermore, Severino *et al.* (2014) reported that modified chitosan based coating containing the nanoemulsion of carvacrol exhibited strong antibacterial activity against *E. coli* inoculated in green bean samples during the storage period [23]. On the other hand, Wang *et al.* (2011) employed chitosan-cinnamon and chitosan-clove EO films, reporting their significant antimicrobial effects against *E. coli*, while the chitosan-star anise EO film showed no antibacterial activity in the mentioned research [24]. On the same note, Shoja Gharehbagh *et al.* (2017) investigated the antibacterial effects of chitosan films incorporated with 2% *Zataria multiflora* EO, and the growth inhibition zone of *E. coli* was reported to be 27.7 millimeters [25]. In line with these findings, Hamedi *et al.* (2019) reported that chitosan/alginate films loaded with antibacterial Thyme oil nanoemulsions could decrease the growth of *E.coli* in vitro [26].

Table 2 shows the mean reduction rate of the *E. coli* O_{157}:H_7 count in various treatments. Correspondingly, the maximum reduction rate was observed with Ch-CNF-NEOs (0.8 log CFU/g) as opposed to the control group. Moreover, the incorporation of the EOs into the chitosan films could inhibit microbial growth more effectively compared to pure chitosan. This finding is in congruence with several studies in this regard [17,20]. Previous findings have also confirmed the antibacterial properties of NTEO and NBPEO against *E. coli*. [27, 28, 29].

Table 2: Mean reduction rate of *E. coli* O_{157}:H_7 counts (Log CFU/g) in Comparison of treatments Groups during 12 days of storage at 4±1°C (Mean Difference I-J)

Group (I)	Group (J)		
	CON	Ch-CNF	Ch-CNF-NEOs
CON		0.44 *	0.80 *
Ch-CNF			0.34 *

$P < 0.05$ *

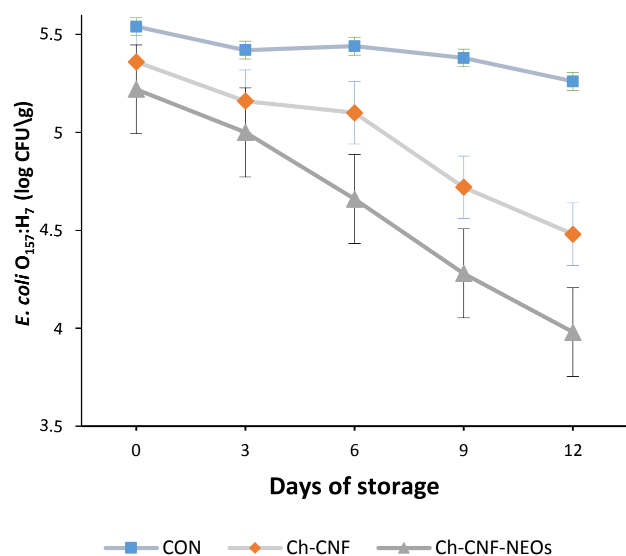

Figure 1: Effects of Treatments on *E. coli* O_{157}:H_7 Growth in Hamburger during Storage at 4±1°C for 12 Days

–Each point representing mean ± SD

4. Conclusion

According to the results, the Ch-CNF and Ch-CNF-NEO films exerted antibacterial effects against the inoculated *E. coli* O_{157}:H_7 in the hamburger samples during 12 days of storage at refrigerated temperature. In addition, Ch-CNF-NEOs had the most significant inhibitory effects on the bacterial growth. Therefore, it could be concluded that edible films composed of chitosan/CNF, NBPEO, and NTEO are novel approaches to enhancing the microbial safety of perishable food products (e.g., meat and meat products), and it is proposed that Ch-CNF-NEO films be practically used in the meat industry.

Authors' Contributions

B.S., performed the laboratory tasks, S.Kh., designed the study M.H., revised the manuscript, and M.A., performed the statistical analysis of the data.

Conflict of Interest

The Authors declare that there is no conflict of interest.

Acknowledgments

Hereby, we extend our gratitude to Ferdowsi University of Mashhad, Mashhad, Iran for the financial support of this study (Grant No. 3/3/48555). We would also like to thank Mrs. S. Khajenasiri for the laboratory support and her kind cooperation.

References

1. Lagos MJB, Sobral PJdA. Application of Active Films with Natural Extract for Beef Hamburger Preservation. *Cienc Rural.* 2019; 49(1).

2. Lahou E, Wang X, De Boeck E, Verguldt E, Geeraerd A, Devlieghere F, *et al.* Effectiveness of Inactivation of Foodborne Pathogens During Simulated Home pan Frying of Steak, Hamburger or Meat Strips. *Int J Food Microbiol.* 2015; 206: 118-29.

3. Stratakos AC, Grant IR. Evaluation of the Efficacy of Multiple Physical, Biological and Natural Antimicrobial Interventions for Control of Pathogenic Escherichia Coli on Beef. *Food Microbiol.* 2018; 76: 209-18.

4. Boqvist S, Fernström LL, Alsanius BW, Lindqvist R. *Escherichia coli* O_{157}: H_7 Reduction in Hamburgers with Regard to Premature browning of Minced Beef, Colour Score and Method for Determining Doneness. *Int J Food Microbiol.* 2015; 215: 109-16.

5. Raji F, Khanzadi S, Hashemi M, Azizzadeh M. Effect of Chitosan Coating Nano-emulsion Containing *Zataria multiflora* and *Bunium persicum* Essential Oils on *Escherichia Coli* O_{157}: H_7 in Vacuum-packed Rainbow Trout Fillet. *J Human Environ Health Promot.* 2019; 5(1): 21-5.

6. Khezrian A, Shahbazi Y. Application of Nanocompostie Chitosan and Carboxymethyl Cellulose Films Containing Natural Preservative Compounds in Minced Camel's Meat. *Int J Biol Macromol.* 2018; 106: 1146-58.

7. Celebi H, Kurt A. Effects of Processing on the Properties of Chitosan/Cellulose Nanocrystal Films. *Carbohydr.* 2015; 133: 284-93.

8. Mechergui K, Coelho JA, Serra MC, Lamine SB, Boukhchina S, Khouja ML. Essential Oils of Origanum Vulgare l. Subsp. Glandulosum (desf.) Ietswaart from Tunisia: Chemical Composition and Antioxidant Activity. *J Sci Food Agric.* 2010; 90(10): 1745-9.

9. Aminzare M, Hashemi M, Hassanzad Azar H, Amiri E, Abbasi Z. Antibacterial Activity of Corn Starch Films Incorporated with *Zataria multiflora* and *Bonium persicum* Essential Oils. *Annu Rev Cancer Biol.* 2017; 19(1): 1-9.

10. Raeisi S, Sharifi Rad M, Quek SY, Shabanpour B, Sharifi Rad J. Evaluation of Antioxidant and Antimicrobial Effects of Shallot (Allium Ascalonicum l.) Fruit and Ajwain (Trachyspermum ammi (l.) Sprague) Seed Extracts in Semi-Fried Coated Rainbow Trout (Oncorhynchus Mykiss) Fillets for Shelf-Life Extension. *Lebenson Wiss Technol.* 2016; 65: 112-21.

11. Gandomi H, Abbaszadeh S, JebelliJavan A, Sharifzadeh A. Chemical Constituents, Antimicrobial and Antioxidative Effects of T rachyspermum ammi Essential Oil. *J Food Process Preserv.* 2014; 38(4): 1690-5.

12. Rashidi L, Khosravi Darani K. The Applications of Nanotechnology in Food Industry. *Crit Rev Food Sci Nutr.* 2011; 51(8): 723-30.

13. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, et al. Formation and Stability of Oil-in-Water Nanoemulsions Containing Rice Bran oil: in Vitro and in Vivo Assessments. *J Nanobiotechnology.* 2011; 9(1): 44.

14. Donsì F, Annunziata M, Vincensi M, Ferrari G. Design of Nanoemulsion-Based Delivery Systems of Natural Antimicrobials: Effect of the Emulsifier. *J Biotechnol.* 2012; 159(4): 342-50.

15. Donsì F, Annunziata M, Sessa M, Ferrari G. Nanoencapsulation of Essential Oils to Enhance their Antimicrobial Activity in Foods. *Lebenson Wiss Technol.* 2011; 44(9): 1908-14.

16. Hashtjin AM, Abbasi S. Nano-Emulsification of Orange Peel Essential Oil Using Sonication and Native Gums. *Food Hydrocoll.* 2015; 44: 40-8.

17. 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 Chem.* 2010; 122(1): 161-6.

18. Azizian A, Khanzadi S, Hashemi M, Azizzadeh M. Inhibitory Effect of Nano-gel/Emulsion of Chitosan Coating Incorporated with Ziziphora Clinopodioides Essential Oil and Nisin on *Escherichia coli* O_{157}: H_7 Inoculated in Beef at Cold Storage Condition. *J Nutr Fasting Health.* 2019; 7(2): 103-9.

19. Kılıç B, Şimşek A, Claus J, Karaca E, Bilecen D. Improving Lipid Oxidation Inhibition in Cooked Beef Hamburger Patties during Refrigerated Storage with Encapsulated Polyphosphate Incorporation. *LWT.* 2018; 92: 290-6.

20. Zivanovic S, Chi S, Draughon AF. Antimicrobial Activity of Chitosan Films Enriched with Essential Oils. *J Food Sci.* 2005; 70(1): M45-51.

21. Vardaka VD, Yehia HM, Savvaidis IN. Effects of Citrox and Chitosan on the Survival of *Escherichia Coli* O_{157}: H_7 and Salmonella Enterica In Vacuum-Packaged Turkey Meat. *Food Microbiol.* 2016; 58: 128-34.

22. Shekarforoush SS, Basiri S, Ebrahimnejad H, Hosseinzadeh S. Effect of Chitosan on Spoilage Bacteria, *Escherichia coli* and *listeria monocytogenes* in Cured Chicken Meat. *Int J Biol Macromol*. 2015; 76: 303-9.

23. Severino R, Ferrari G, Vu KD, Donsì F, Salmieri S, Lacroix M. Antimicrobial Effects of Modified Chitosan Based Coating Containing Nanoemulsion of Essential Oils, Modified Atmosphere Packaging and Gamma Irradiation against *Escherichia coli* O_{157}: H_7 and *Salmonella typhimurium* on Green Beans. *Food Control*. 2015; 50: 215-22.

24. Wang L, Liu F, Jiang Y, Chai Z, Li P, Cheng Y, *et al.* Synergistic Antimicrobial Activities of Natural Essential Oils with Chitosan Films. *J Agric Food Chem*. 2011; 59(23): 12411-9.

25. Shoja Gharehbagh S, Khanjari A, Yeganmohammadi Davaji M, Akhondzadeh Basti A. The Use of Chitosan and Whey Protein Isolate Edible Films Incorporated with *Zataria multiflora* Boiss. Essential Oil as an Active Packaging Ingredient against Some Common Foodborne Bacteri. *J Food Biosci Technol.* 2017; 7(1): 41-8.

26. Hamedi H, Moradi S, Tonelli AE, Hudson SM. Preparation and Characterization of Chitosan–alginate Polyelectrolyte Complexes Loaded with Antibacterial Thyme Oil Nanoemulsions. *Appl Sci.* 2019; 9(18): 3933.

27. Oroojalian F, Kasra Kermanshahi R, Azizi M, Bassami MR. Phytochemical Compositon of the Essential Oils from Three Apiaceae Species and Their Antibacterial Effects on Food-Borne Pathogens. *Food Chem*. 2010; 120(3): 765-70.

28. Goudarzi GR, Saharkhiz M, Sattari M, Zomorodian K. Antibacterial Activity and chemical Composition of Ajowan (Carum copticum Benth. & Hook) Essential Oil. *J Agric Sci Technol*. 2011; 13: 203-8.

29. Noshad M, Hojjati M, Behbahani BA. Black Zira Essential Oil: Chemical Compositions and Antimicrobial Activity against the Growth of Some Pathogenic Strain Causing Infection. *Microb Pathog*. 2018; 116: 153-7.