Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Nutrition & Food Security Research Center

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2019; 4(4): 236-242 Website: jnfs.ssu.ac.ir

Combined Effect of Chitosan-Based Edible Film Containing Oregano Essential Oil on the Shelf-Life Extension of Fresh Chicken Meat

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ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received:23 May 2018
Revised: 4 Jul 2018
Accepted: 27 Sep 2018

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ABSTRACT

Background: The aim of this study was to investigate the effects of chitosan coatings, oregano essential oil, and their combination on microbial quality of chicken fillet during 12 days in refrigerator temperature. **Methods:** Oregano oil was extracted by water distillation and then different concentrations of oregano oil (1% and 2%) were used for film preparation. Microbiological tests were carried out on the chicken fillet samples stored in 4 °C at different intervals including days 0, 3, 6, 9, and 12. **Results:** Treated groups with chitosan and oregano oil affected the increase rate of aerobic plate counts, coliform counts, and total psychrophilic counts significantly compared to the control group across the entire storage period ($P < 0.05$). Chitosan-based edible film containing oregano oil inhibited microbial growth on chicken fillet. Microbial populations were reduced by 2.14-3.53 log CFU/g in groups treated with chitosan and oregano oil. Our results revealed that incorporation of oregano essential oil at 2% concentration had the highest inhibitory effect on spoilage microorganisms in coated chicken fillet during 12 days of storage at refrigerator. **Conclusion:** Generally, application of oregano essential oil at 2% concentration had the potential to enhance safety and shelf-life of chicken fillet.

Keywords: *Chitosan film; Oregano oil; Chicken meat; Microbial quality; Shelf-life*

Introduction

Nowadays, chicken meat is consumed widely all over the world. Chicken meat, as a raw foodstuff with no process for producing or keeping, is vulnerable to spoilage and can be an almost perfect medium for microbial growth including both spoilage and pathogenic microorganisms. Therefore, food producing factories have desire to extend shelf life of the chicken meat and its products by new packaging methods (de Azeredo, 2013). Furthermore, today's environmental contamination has increased due to the use of plastic packaging coatings and the antimicrobial resistance issue (Bohlmann, 2005). These factors have led the food production factories to use biodegradable coatings, including chitosan. Biodegradable films are thin layers of edible materials formed as coatings on foodstuffs, which can optimize the moisture and atmosphere of the food product and prevent entry of foreign materials (Dutta *et al.*, 2009). However, biodegradable films can have antimicrobial and

This paper should be cited as: Karimnezhad F, Razavilar V, Anvar AA, Dashtgol Sh, Pilehvar Zavareh A. ***Combined Effect of Chitosan-Based Edible Film Containing Oregano Essential Oil on the Shelf-Life Extension of Fresh Chicken Meat.*** *Journal of Nutrition and Food Security (JNFS)*, 2019; 4 (4): 236-242.

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[DOR: 20.1001.1.24767417.2019.4.4.2.6]
[DOI: 10.18502/jnfs.v4i4.1720]

antioxidant properties, which are enhanced by natural compounds such as essential oil (Pranoto *et al.*, 2005). Chitosan is a natural polysaccharide polymer, derived by deacetylation of chitin (Elsabee and Abdou, 2013). It is also considered as the generally recognized as safe (GRAS) by United States Food and Drug Administration (Kerch, 2015). Chitosan has been proved as a wide spectrum of antimicrobial activity against gram positive/gram negative bacteria and fungi. Biocompatibility, nontoxicity and biodegradability properties of chitosan make it suitable for the food preservation (Zimoch-Korzycka *et al.*, 2016). Ample evidence shows that chitosan can be used as edible films and coatings in various meat products (Lekjing, 2016, Petrou *et al.*, 2012).

Recently, food producing factories have a positive attitude to use the extracts and essential oils from herbs to improve the foods' sensory parameters and shelf life (Botsoglou *et al.*, 2003). Previous studies reported that oregano essential oil had high activity against microorganisms (Chouliara *et al.*, 2007). Considerable antimicrobial and antioxidant activity are related to phenolic monoterpens and carvacrol components (Alsaiqali *et al.*, 2016). Recently, application of chitosan and essential oils has increased in food coating to control the microbial populations and improve the shelf life of meat products. Various studies investigated the shelf life extension by chitosan as a dipping agent incorporation of oregano essential oil on modified atmosphere packaging (MAP) for the extension of fresh poultry meat shelf life (Petrou *et al.*, 2012). Pomegranate juice dipping and chitosan enriched with Zataria multiflora had inhibitory effects on the microbial growth and chemical changes in chicken breast meat (Bazargani-Gilani *et al.*, 2015). Lekjing showed the preservative effects of chitosan-based coating with clove oil on quality and shelf life of pork sausage (Lekjing, 2016).

Considering the above, the objective of this study was to evaluate the combined effect of chitosan-based edible film containing oregano essential oil on microbiological parameter of chicken meat.

Materials and Methods

Plant material and preparation of oregano essential oil: Origanum vulgare subsp. plants were collected from Gorgan City, Iran, from July to September 2016. Identification of voucher specimen was carried out by Tehran University Herbarium, Iran. Essential oil was obtained from 50 g of dried plant material by hydro-distillation using Clevenger's apparatus for 3 h at 70 °C. Isolated oil dried over anhydrous sodium sulphate (Merck, Germany). Oregano oil was recovered and stored in screw capped bottles at 4 °C.

Preparation of edible films: A solution of chitosan (Sigma-Aldrich, USA) was provided by dissolving chitosan of high molecular (800,000 cps; >75% deacetylation degree) weight in powder in 100 ml of acetic acid (Merck, Germany) and stirring at 45 °C for 3 h to obtain the final concentration 2% (w/v). The chitosan solution was filtered using a Whotman No. 3 filter paper and continued by vacuum filtration to remove any undissolved particles. To prepare chitosan solution with essential oil, 30% w/v of Glycerol (Sigma-Aldrich, USA) and 0.25% w/v of Tween 80 were added in film formation solutions. The pH of solution was adjusted to 5.8 by NaOH (Merck, Germany). The essential oil of oregano was added to the chitosan solution to obtain the final concentration of 1% and 2% (w/v). To prepare an emulsion, the solution was homogenized (IKA T25 basic, Staufen, Germany) at 8000 rpm for 3 min. The mixture was cast onto a flat polytetrafluoroethylene casting plate and dried at room temperature for at least 36 h. Dried films were kept under conditions 25 °C and 50% RH for 48 h (Moradi *et al.*, 2011).

Preparation of chicken meat samples: Fresh chicken fillet was provided by a slaughter house in Tehran province, Iran (samples weighing 400 g, 15 cm × 7 cm each). All meat samples were packaged by chitosan based films for further treatment. The samples were assigned and treated as follows:

1) Chitosan group: chicken fillet coated with chitosan free essential oil.

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[DOI: 10.18502/jnfs.v4i4.1720]

2) & 3) Treated groups with oregano essential oil: chicken fillet coated with chitosan containing 1% and 2% concentrations of oregano, respectively.

4) Control group: Chicken fillet with no package.

All meat samples were kept at 4 °C for a period of 12 days. Sampling was carried out on 0, 3, 6, 9, and 12 days of storage.

Microbiological examinations: Aerobic plate counts (APC), coliform counts (CC), and total psychrophilic counts (TPC) were determined as follows: a 10% suspension of each sample was prepared by homogenizing in peptone water (Merck, Germany) using stomacher (IUL Instrument, Spain). Serial tenfold dilution was made from 10^{-2} to 10^{-6} in sterile peptone water diluent. Later, 0.1 ml of each dilution was inoculated into selective agar plates. Aerobic plate counts were determined by the spread -plate method using plate count agar (Merck, Germany) and incubated (Binder, Germany) at 37 °C for 24 h and 48 h (Association, 1992). Coliform counting was assessed on the basis of pour plate method; the sample was spread into Violet Red Bill agar (Merck, Germany) and incubated at 35 ± 2 °C for 48 h. Suspected colonies were cultured into the Brilliant Green Broth (Merck, Germany) following the incubation at 35 ± 2 °C for 24-48 h (Association, 1992). Furthermore, TPC was done similar to APC but the plates were incubated at 7 °C for 7-10 days (Association, 1992). The counts were showed as log CFU/g.

Data analysis: All tests were done three times. Data were presented as mean ± Standard Deviation (SD) and analyzed using SPSS (SPSS Inc, Chicago, IL. v. 22.0). Significance levels ($P < 0.05$) were assessed by ANOVA and supplementary Tukey HSD tests.

Results

Changes in APC: **Table 1** shows the number of chicken fillet APC in the control (untreated) and treated groups during the storage period for 12 days. The initial APC counts ranged from 3.91 to 8.32 log CFU/g and the counts increased in the control and treated groups throughout the storage time. The increase rate of aerobic bacteria counts in groups of chicken fillet treated with chitosan, with 1% oregano essential oil, as well as with chitosan and 2% oregano were significantly lower comparable to the control group. Statistically significant differences were observed for the APC of chicken fillet on 3, 6, 9, and 12 days of storage period between the control and treated groups (two different concentrations of oregano essential oil).

Changes in TPC: **Table 2** shows the TPC rates of chicken fillet in the control and treated groups during the storage. The total psychrophilic counts ranged from 4.2 ± 0.13 to 8.65 ± 0.28 log CFU/g. We observed that the counts gradually increased the control and chitosan groups during the storage period. However, the increase levels were lower in the treated samples than the control sample. Statistically significant differences were observed for the TPC of chicken fillet between the control and treated groups across the entire storage periods.

Changes in CC: **Table 3** represents the coliform count of chitosan fillet in the control and treated groups during the storage period. The numbers of total coliform bacteria were in the range of 1.89 ± 0.1 to 5.62 ± 0.16 log CFU/g and the count consistently increased in the control and treated groups throughout the storage duration. However, the rate of increase was slower for chicken fillet treated by chitosan enriched with 1% and 2% concentrations of oregano essential oil compared to the control group. In group of fillet chitosan treated with 2% oregano, CC decreased in days 3 and 9, while it increased in days 6 and 12 throughout the storage period. Since the CC levels increased gradually in the group treated with 1% essential oil. Statistically significant differences were observed in chicken fillet CC in days 3, 6, 9, and 12 of storage between the control and treated groups.

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[DOR: 20.1001.1.24767417.2019.4.4.2.6]
[DOI: 10.18502/jnfs.v4i4.1720]

Table 1. Effect of edible chitosan film, oregano essential oil, and their combinationon APC of fresh chicken meat stored at 4 °C

Day	Control	Chitosan with 1% oregano essential oil	Chitosan with 2% oregano essential oil	Chitosan
0	4.22* ± 0.27 ^{Aa}	3.98 ± 0.12 ^{Aa}	3.91 ± 0.17 ^{Aa}	4.01 ± 0.09 ^{Aa}
3	5.03 ± 1.05 ^{Aab}	4.24 ± 0.17 ^{Bab}	4.02 ± 0.10 ^{Ba}	4.52 ± 0.27 ^{ABb}
6	6.16 ± 0.42 ^{Ab}	4.23 ± 0.05 ^{Bb}	4.11 ± 0.09 ^{Ba}	5.21 ± 0.24 ^{Cb}
9	6.95 ± 0.9 ^{Abcd}	4.9 ± 0.07 ^{Bc}	4.68 ± 0.46 ^{Bab}	6.17 ± 0.17 ^{Cc}
12	8.32 ± 0.26 ^{Ad}	6.18 ± 0.13 ^{Bd}	5.23 ± 0.13 ^{Cb}	7.34 ± 0.18 ^{Dd}

a-d: means with different lowercase letters in the same column are significantly different ($P < 0.05$), A-D: means with different capital letters in the same row are significantly different ($P < 0.05$), *: log CFU/g

Table 2. The effect of edible chitosan film, oregano essential oil, and their combination on TPC of fresh chicken meat stored at 4 °C

Day	Control	Chitosan with 1% oregano essential oil	Chitosan with 2% oregano essential oil	Chitosan
0	4.59* ± 0.20 ^{Aa}	4.33 ± 0.1 ^{BCa}	4.20 ± 0.13 ^{Ba}	4.45 ± 0.08 ^{ACa}
3	5.21 ± 0.23 ^{Ab}	4.45 ± 0.15 ^{Ba}	4.24 ± 0.07 ^{Bac}	4.72 ± 0.16 ^{Cab}
6	6.68 ± 0.19 ^{Ac}	4.71 ± 0.20 ^{Bb}	4.31 ± 0.07 ^{Cab}	5.22 ± 0.25 ^{Db}
9	7.35 ± 0.16 ^{Ad}	4.86 ± 0.15 ^{Bc}	4.53 ± 0.13 ^{Cbc}	6.48 ± 0.27 ^{Dc}
12	8.65 ± 0.28 ^{Ae}	5.68 ± 0.18 ^{Bd}	5.12 ± 0.12 ^{Cd}	8.32 ± 0.25 ^{Ad}

a-e: means with different lowercase letters in the same column are significantly different ($P < 0.05$), A-D: means with different capital letters in the same row are significantly different ($P < 0.05$), *: log CFU/g

Table 3. Effect of edible chitosan film, oregano essential oil, and their combinationon CC of fresh chicken meat stored at 4 °C

Day	Control	Chitosan with 1% oregano essential oil	Chitosan with 2% oregano essential oil	Chitosan
0	2.14* ± 0.14 ^{Aa}	1.89 ± 0.10 ^{Ba}	1.96 ± 0.07 ^{Ba}	2.00 ± 0.01 ^{ACa}
3	3.00 ± 0.11 ^{Ab}	2.08 ± 0.08 ^{BCb}	1.78 ± 0.42 ^{Babcd}	2.38 ± 0.09 ^{Ca}
6	4.17 ± 0.17 ^{Ac}	2.51 ± 0.08 ^{Bc}	2.65 ± 0.04 ^{Bb}	2.94 ± 0.12 ^{Cb}
9	4.18 ± 0.16 ^{Ad}	2.95 ± 0.13 ^{Bcd}	2.23 ± 0.04 ^{Cc}	3.69 ± 0.19 ^{Db}
12	5.62 ± 0.16 ^{Ae}	3.01 ± 0.07 ^{Bd}	2.51 ± 0.07 ^{Cd}	4.97 ± 0.11 ^{Dc}

a-e: means with different lowercase letters in the same column are significantly different ($P < 0.05$), A-D: means with different capital letters in the same row are significantly different ($P < 0.05$), * : log CFU/g

Discussion

The present experiments showed that the combination of chitosan and oregano essential oil (1% and 2%) inhibited the growth of microbial populations. Application of chitosan film including 2% concentration of oregano had the most antimicrobial effect on chicken fillet. The films reduced the APC, TPC, and CC, which it has the potential to preserve chicken fillet. Similarly, Petrou et al. revealed that chicken breast meat treated with chitosan 1.5% w/v and oregano oil 0.25% v/w under MAP had the most effective results regarding the TPC growth suppression with a shelf life of 14 days (Petrou *et al.*, 2012). In other studies, Chouliara et al. demonstrated that incorporation of MAP and oregano essential oil 1% had a stronger preservative effect on suppression of the microflora in chicken breast meat with concentration of 0.1% oregano oil (Chouliara *et*

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al., 2007). Petrou et al. reported that combination of chitosan and oregano into chicken breast meat samples reduced the numbers of TVC by 3-4 log CFU/g compared to the control group (Petrou *et al.*, 2012). Therefore, these results are in agreement with our study, which showed that application of an essential oil (containing oregano 1% and 2%) with chitosan film in the chicken fillet decreased the levels of APC by 2.14 and 3.09, respectively. Likewise, our results are in agreement with those of Tsigarida, et al., who reported reduction of TVC in beef meat fillet by 2-3 log CFU/g when oregano essential oil was added at the concentration of 0.8% (Tsigarida *et al.*, 2000). Lekjing indicated that coating with a mixture of 2% chitosan and 1.5% clove oil showed the most effective results in inhibiting the TVC growth for 25 days of pork sausage storage (Lekjing, 2016). In another study, Georgantelis et al. demonstrated that incorporation of rosemary extract and chitosan reduced the TVC by 1-2 log CFU/g on fresh pork sausage (Georgantelis *et al.*, 2007).

Beverya et al. indicated that acetic acid and lactic acid chitosan coatings could be used to control *L.monocytogenes* on ready- to - eat roast beef. They revealed that chitosan coatings had less effectiveness in reducing *L. monocytogenes* compared to chitosan coating enriched with acetic acid and lactic acid (Beverlya *et al.*, 2008). In the present study, the chitosan-based edible film with oregano essential oil was more effective than the chitosan alone in controlling the microbial loads in chicken fillet. Zivanovic et al. reported that addition of oregano essential oil into the chitosan film decreased the numbers of *L. monocytogenes* and *E. coli* by 3.6 - 4 and 3 logs CFU/g, respectively. However, pure chitosan films reduced *L. monocytogenes* by 2 logs CFU/g (Zivanovic *et al.*, 2005). In our study, chitosan with oregano essential oil had higher antimicrobial effects than chitosan applied alone. Combination of chitosan with 1% and 2% concentrations of oregano essential oil reduced the TPC by 2.97 and 3.53 logs CFU/g, respectively. The counts of coliform bacteria decreased by 2.61 and 3.11 log CFU/g in the chicken fillet samples treated with chitosan and essential oil containing 1% and 2% oregano, respectively. This is in agreement with the results of a study by Chouliara et al., who reported that use of MAP and oregano oil had a strong effect on suppression of *Enterobacteriacea*. Moreover, MAP and oregano oil 0.1% reduced the *Enterobacteriacea* counts up to 1.8 log CFU/g, while MAP and oregano oil 1% reduced the *Enterobacteriacea* counts more than 6 log CFU/g (Chouliara *et al.*, 2007). These results can be attributed to the inhibitory effect of the combined antimicrobials containing chitosan and oregano essential oil. Chitosan shows a broad spectrum antimicrobial activity against the microorganisms. It acts by changing the permeability of membrane wall that leads to the internal osmosis imbalance, intracellular electrolytes leakage, and finally causes cell death. As a result of chitosan bounding with microbial DNA and peptidoglycan hydrolysis, chelating metals are exhibited as other mechanisms for antimicrobial effects of chitosan (Goy *et al.*, 2009). The antibacterial action of oregano includes disturbance of the cytoplasmic membrane, disruption of the protein motive force, electron flow, and active transport, as well as coagulation of the cell content (Burt, 2004).

Conclusion

Results of the present study demonstrated that combination of both chitosan and oregano essential oil inhibited the microbial growth on chicken fillet**.** Chitosan film containing oregano oil 2% had the best effect on chicken fillet shelf life during 12 days of storage at refrigerator.

The investigations of physicochemical and sensory parameters of coated chicken fillet can be considered for further studies.

Acknowledgements

The author thanks the staffs of the Food Science and Technology and Medicinal Plants Research Centers in Islamic Azad University, Science and Research Branch, Tehran, Iran, for their important technical supports.

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[DOR: 20.1001.1.24767417.2019.4.4.2.6]

[DOI: 10.18502/jnfs.v4i4.1720]

Authors' contribution

All authors contributed to design, data gathering and analysis and the final version of the manuscript.

Conflict of interest

There is not any conflict of interest.

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[DOR: 20.1001.1.24767417.2019.4.4.2.6]

[DOI: 10.18502/jnfs.v4i4.1720]