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Original Article

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Comparison The Effect of Carboxymethyle Cellulose Films Containing Thymus vulgaris and Zataria multiflura on Shelf Life of Chicken Meat

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Abstract

Poultry meat is a kind of perishable food. The growth of pathogenic microorganisms may occur in chicken meat during storage in the refrigerator. Microbial growth causes a serious hazard to the safety of the food consumer. The aim of this study was to compare antimicrobial properties of Thymus vulgaris (TEO) and Zataria multiflora essential oil (ZEO) and the effect of carboxymethyl cellulose (CMC) films containing essential oil on shelf life of chicken meat during 9 days at 4° C. Essential oil was extracted by distillation. At first, antimicrobial activities of CMC film containing essential oil and control films were analyzed by disc diffusion assay. After that, three treatments of chicken fillets including untreated-control (C), coating carboxymetyl cellulose (CMC), and CMC with 2.4% Zataria multiflora essential oil (CMC-Z) were prepared. The microbial shelf life of treatment were determined in 3 days interval at 4 ° C. The results showed that antimicrobial properties of Thymus vulgaris and Zataria multiflura essential oil, ZEO containing film had efficient inhibitory effect compared to TEO, thus film incorporated with ZEO was selected for shelf life studies. Also the results revealed that total viable count (TVC) population of fillets increased during shelf life and exceeded 6.81 log cfu g -1 for CMC sample on day 8. For CMC-Z treatments, this deadline was achieved after 9 days. Coating chicken meat sample with CMC film could decrease TVC population compared to the control sample ($p<0.05$). The results showed that the use of ZEO in chicken meat as antimicrobial compound caused a delay in microbial putrefaction process.

Keywords: Thymus vulgaris, Zataria multiflora, Antimicrobial Coating, Shelf Life, Chicken Meat

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Introduction

Poultry products are considered as a good source of protein in human nutrition due to high protein value, low fat, and low cost. However, the chicken meat is susceptible to spoilage, even under refrigerated condition. Lipid oxidation causes unpleasant and undesirable odor, taste, texture, and degradation in nutritional value. Also, microbial putrefaction causes a serious hazard to health of the consumer and decreased shelf life (Bazargani Gilani et al.,2015).

Active packaging is one of the new purports in food packaging that eventuate for extending food shelf-life and maintain nutrition value. (Salarbashi et al.,2014).

Recently, an increasing interest has been aroused for edible films and coatings. Edible films and coating have many advantages including biodegradability, good oxygen resistance, availability, low cost and biocompatibility (Ghasemlou et al.,2013).

Carboxymethyl cellulose (CMC), as a non-toxic and water-soluble cellulose derivative, has good film forming properties (Dashipour et al.,2015).

Edible films are known as a good carrier for active components such as antimicrobial and antioxidant agents, which can make them active packaging. Essential oils can be used as the antimicrobial agent into edible films matrices (Broumand et al.,2011). Due to their phenolic content, essential oils, unlike chemical retentive, can increase the quality and safety of food by inhibition of the bacterial growth and reduction of lipid oxidation (Arts et al.,2007).

Thymus vulgaris and zataria multiflora Boiss, known as " Avishane bagi " and " Avishane shirazi" in Persian, respectively, belong to the Labiates family. They have known antioxidant, antibacterial, antifungal, and medicinal properties. Thymol and carvacrol are the main phenolic compound of these plants (Saei-Dehkordi et al.,2010; Shahbazi et al.,2014).

The aim of this study was to extract Thymus vulgaris and Zataria multiflora Boiss essential oils and compare their antimicrobial activities in in vitro studies and then evaluate the effect of CMC films incorporated Zataria multiflora essential oil on the microbial shelf life of chicken meat during storage at the refrigerator.

Materials and Method

Materials

Carboxymethyl cellulose (CMC) was purchased from Pharayandsazan Arian Persian Co. Avishane shirazi and Avishane bagi were purchased from the local tradition market. Glycerol, Tween 80 (analytical grade), Brain Heart Infusion (BHI) and other medium were provided from Merck Co.

Extraction of essential oil

Dried plants of Thymus vulgaris or zataria multiflora were powdered, then plants powder were extracted to water-distillation for 3h in a Clevenger. The oils were separated from the water phase and stored in dark vial at 4 ° C until the experiments (Gouveia et al.,2016).

Preparation of films

CMC film was provided by dissolving 1g of CMC in 100 ml (1% w/v) of distilled water at 70 °C for 45 min on magnetic stirrer, to form a smooth and clear solution. Then film solutions were casted at the center with a 15 cm diameter and 2 cm height of glass plates. Next, the film solutions were dried at 30 °C for 25h, then film was exerted as the control film. The essential oil containing films were prepared by adding Zataria multiflora essential oil to clear solution of CMC at concentrations of 1.6, 2.4 and 3.2 % (v/v). Tween 80 was applied as an emulsifier (based on essential oil) and the solution was homogenized (IKA T25-Digital Ultra Turrax, Staufen, Germany) at 13,500 rpm for 4 min. Finally, emulsion was cooled at 55 °C to remove air bubble. Film solution was casted at the center on glass plates, and dried at 30 °C for 30 h. Dried film was peeled off from the plate and stashed in desiccators (containing saturated magnesium nitrate solution) at 53% relative humidity (RH) and 25 °C (Dashipour et al.,2015).

Evaluation of antimicrobial activity of film Bacterial strains

Staphylococcus aureus ATCC 25923, Escherichia coli PTCC 1399, Salmonella enteritidis ATCC 1231, Bacillus cereus ATCC 1274 and Pseudomonas aeroginosa ATCC 1430 were used, taken from the Iranian Research Organization for Science and Technology (Tehran-Iran). They were grown in Brain Heart

Infusion Broth (BHI Broth) at 37 ° °C for 24h before the essay.

Antimicrobial Assay

Disk diffusion assay was used to determine the antimicrobial activity of treatment. The films were cut into circle shapes (disk) with a 6mm diameter under aseptic condition and then it was placed on Muller-Hinton plate, previously seeded with 100 μl of 24h broth culture containing approximately 107 cfu ml-1 of bacteria. These plates were incubated at 37 °C for 24 h. The clear diameter of the area of inhibition around the disk film was determined (Shojaee-Aliabadi et al.,2013).

Sample preparation

Fresh chicken meat fillets (0.95-0.97 water activity, 79.24% moisture content,) were provided from local poultry and cut into dimensions 8×3×2 cm (treatment weighting ca.50g). The prepared films were wrapped around chicken fillets. Three sample groups were provided: control (aerobic packaging), carboxymethyle cellulose (CMC), CMC film containing zataria multiflora essential oil (CMC-Z). Chicken filets were packed in polyethylene pouches and kept at 4 °C for 9 days (Chouliara et al.,2007).

Microbiological analysis

Chicken treatments (10 g) was taken from each treatment at aseptic condition and were conducted to 90 ml of sterile 0.75% physiological serum solution, then it was mixed and homogenized. They were 7-fold tube diluted and enumeration of bacteria. Total viable count (TVC) was performed using Plate Count Agar (PCA), and incubation at 37 ° C for 3 days. Microbial count was reported as log10 cfu gr -1 (colony forming units).

Statistical Analysis

The data treatments were three replicated and was executed statistical analysis using the SPSS software version 20. Mean and standard deviations obtained from the tests were calculated. For mean comparison, ANOVA was done following by Duncan test. General Linear Model (GLM) was used to compare data on different days.

Table1: Antimicrobial activities (Mean ±SE*) of thymus vulgaris and zataria multiflora essential oil

Inhibition zone (mm)					
Parameter	**S. aureus**	**B.cereus.**	**E.coli**	**S.enteritidis**	**P.aeroginosa**
Thymus vulgaris	41.00±1.73[a]	40.00 ±0.57[a]	21.00±0.57[a]	19.16±0.44[a]	12.00±0.76[a]
Zataria Multiflura	30.00±0.57[b]	43.00±0.88[b]	30.33±0.88[b]	26.00±0.57[b]	30.66±0.88[b]

**SE: Standard Error*

Values with different low letters in all column are significantly different ($p<0.05$)

Results and Discussion

Antimicrobial activity

Table 1 shows the results of antimicrobial properties TEO and ZEO against tested bacteria The results of inhibition zone (mm) indicated that P.aeroginosa had the highest resistance to tested essential oils, however, S. aureus was the most sensitive bacteria ($p<0.05$). ZEO showed more efficient inhibitory action on the all tested bacteria compared to TEO, except for S. aureus. In this study, both essential oils had more destructive effect on positive gram bacteria compared to negative ones. The reason can be associated to the cell wall structure differences of the gram positive bacteria containing thick cell wall and high level peptidoglycan and Teichoic acid, whilst gram negative bacteria has thinner cell wall with hydrophilic structure. Peptidoglycan layer in Gram-positive bacteria act as a barrier against certain essential oil. Based on test results, essential oil of zataria multiflora was selected to produce antimicrobial CMC based film.

Table 2 shows the antimicrobial property of CMC films containing different concentrations (1.2, 2.4 and 3.2%) of zataria multiflora essential oil (ZEO) against five pathogenic bacteria.

The control film (with-out ZEO) showed no inhibitory activity against bacteria. CMC film containing ZEO could inhibit pathogenic bacteria growth. P.aerginosa and S.aureus were highly resistant and sensitive bacteria, respectively. The CMC films with 1.6 % of ZEO

Table 2: Antimicrobial activities (Mean ±SD*) of three concentration of CMC-Z

Inhibition zone (mm)					
Parameter	***S. aureus***	***B.cereus.***	***E.coli***	***S.enteritidis***	***P.aeroginosa***
Control	0.00	0.00	0.00	0.00	0.00
CMC-Z 1.6%	18.50±0.86 [a]	17.67 ±0.21[a]	16.50±2.08[a]	17.50±1.44[a]	6.00±0.00[a]
CMC-Z 2.4%	30.00±0.57[b]	43.00±0.88[b]	30.33±0.88[b]	26.00±0.57[b]	30.66±0.88[b]
CMC-Z 3.2%	36.50±1.44[b]	28.30±0.18[b]	27.33±2.68[b]	27.60±0.3[b]	24.43±1.7[b]

**SD: Standard Deviation*
Values with different low letters in all column are significantly different (p<0.05)

had weak inhibitory zone effect on the growth of bacteria. While inhibition zone of films increased as concentration of ZEO increased, there was a significant difference between antimicrobial activities of film containing 2.4 and 3.2% of ZEO (based on t-test). Antimicrobial activity of ZEO can be attributed to its phenolic compounds such as carvacrol, thymol, γ-terpine and p-cymene. Phenolic compounds can disrupt the external membrane of bacteria, and result in enhanced cytoplasmic permeability (Dashipour et al., 2015). Ghasemlu et al. (2013) reported that corn starch film containing zataria multiflora and mentha pulegium essential oils had antibacterial activities and were also most effective on S.aureus, Furthermore the results reported by Hu et al. (2016) and Shojaee-Aliabadi et al. (2013) were in agreement with the results in this study. Addition of essential oil of ZEO improved specification of CMC film. Despite highest antimicrobial activity of film containing content 3.2% of essential oil, the film had a pungent odor and darker color. So, CMC edible film containing 2.4% of ZEO was utilized to assess microbial analyses on chicken meat during storage in the refrigerator.

Microbiological analyses

Initial TVC (day 0) of chicken fillets was 3.87 log cfu g -1 indicative of relatively good quality of chicken meat (Fig. 1). Mesophilic bacteria population of chicken fillet increased during shelf life and exceeded 6 log cfu g -1 for control treatment on day 6. Considered value of 6 log cfu g -1 as the upper acceptability limit for fresh chicken meat, according Veterinary Organization of Iran, (2009), the shelf life of control sample was about 6 days. However, for CMC samples, after CMC-Z treatment, this deadline was achieved after 8 and 9 day of during storage, respectively, which indicating positive effect of CMC films and tested essential oil on protection of meat against bacterial deterioration and improvement shelf life

Therefore, coating chicken fillet treatment with carboxymetyle cellulose including essential oil film could decrease TVC bacteria (aerobic bacteria) compared to the control sample. It can be attributed to antibacterial activities of ZEO and probably to the barrier effect of coating against oxygen permeability as well (Zinoviadou et al.,2009).

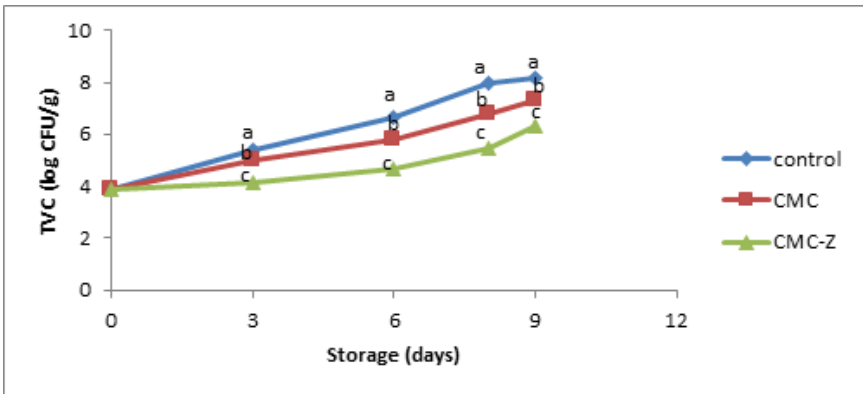

Figure1: Changes in TVC bacteria in raw chicken during storage. a-c, means average values standard deviation, with different low letters are significantly different (p<0.05)

Conclusions

The results showed good antibacterial activities of ZEO and TEO, however, ZEO was a more efficient antimicrobial agent. Microbiological analyses on chicken fillet sample with CMC-Z showed that the addition of ZEO enhanced shelf life of chicken fillets during storage at refrigerated temperature. Therefore, CMC film containing ZEO has potential for antimicrobial packaging for food product systems.

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