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Effect of carboxymethyl cellulose-based active packaging incorporated with *Ziziphora clinopodioide*s extract on the shelf life of chicken fillet

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Abstract

The use of biodegradable polymers, especially in the form of active packaging, has shown promising results in maintaining chicken meat quality and extending its shelf life. This study aimed to evaluate the effect of active packaging based on carboxymethyl cellulose (CMC) and *Ziziphora clinopodioides* extract on the shelf life of chicken fillets during a 12-day refrigerated storage. A complete randomized block design was implemented with five treatments, including uncoated chicken fillets (control), and fillets coated with *Z. clinopodioides* extract at 0.75% and 1.5%, either alone or in combination with CMC as active packaging. The results showed that *Z. clinopodioides* extract significantly prevented microbial spoilage and oxidative deterioration of the fillets in a concentration-dependent manner. The use of CMC facilitated the slow and continuous release of the extract to the fillets, maintaining the effectiveness of the extracts. Fillets with active packaging containing 1.5% extract had the lowest mean ($p < 0.05$) counts of mesophilic bacteria and psychrophilic bacteria (5.00 and 3.90 log CFU/g, respectively), total volatile basic nitrogen (16.45 mg/100g), peroxide value (2.95 meq/kg), and thiobarbituric acid index (0.65 mg MDA/kg), while exhibiting the highest water-holding capacity (53.15%). In conclusion, it was determined that active biodegradable packaging based on CMC containing 1.5% *Z. clinopodioides* extract effectively preserved the quality and extended the shelf life of chicken fillets for at least six days at refrigerator temperature.

Introduction

Despite the development of methods to enhance the shelf life and safety of food products, microbial spoilage and undesirable metabolites produced by microorganisms remain significant challenges in the food industry, particularly for animal products (Amirpour *et al*., 2015). Packaging serves as an effective solution to protect food from undesirable microbial and chemical changes. Global awareness of environmental issues related to the use of synthetic materials in packaging has led to increased interest in biodegradable packaging materials based on biopolymers. Therefore, biodegradable packaging is a growing strategy for addressing sustainability challenges in the food packaging industry (Bajer *et al*., 2020).

Cellulose and its derivatives are biopolymers widely used as food packaging materials. However, cellulose is highly crystalline and insoluble, due to its specific chemical structure. In contrast, carboxymethyl cellulose (CMC), one of its most important derivatives, is water-soluble and forms flexible and strong films. The CMC is non-toxic, non-allergenic, flexible, odorless, tasteless, transparent, and has moderate permeability to moisture and oxygen (Khademi Shurmasti, 2022). Our previous studies have shown that coatings made from CMC in the form of active packaging can serve as carriers for various additives and compounds, such as antimicrobial and antioxidant agents (Ahmadi and Khademi Shurmasti, 2020; Bahrami Feridoni and Khademi Shurmasti, 2020; Ehsan and Khademi Shurmasti, 2022; Sharifi and Khademi Shurmasti, 2022).

Active packaging based on natural active ingredients has garnered significant attention in the past decade. Active packaging is defined as packaging that provides functions beyond traditional protection (Sarfraz *et al*., 2021). These active packages, containing active components, such as antimicrobials, antioxidants, oxygen scavengers, carbon dioxide emitters/absorbers, moisture regulators, flavor release agents, and absorbers, can delay or prevent microbial, enzymatic, and oxidative spoilage (Ahmed *et al*., 2017).

Numerous studies reported the antioxidant and antibacterial properties of various edible coatings and biodegradable packaging with bioactive components derived from plant extracts and essential oils, such as citral and cinnamon (Sarfraz *et al*., 2021), *Zataria multiflora* Boiss and *Cuminum cyminum* L. (Zafarmand Kashani and Khademi Shurmasti, 2021), *Cymbopogon citratus* (Mardani Kiasari and Khademi Shurmasti, 2020), *Satureja hortensis* (Khademi Shurmasti*et al*., 2021), and *Eryngium caucasicum* (Golmohammadi and Khademi Shurmasti, 2019) extracts on the shelf life of chicken fillets. However, it has been reported that when antimicrobial compounds are used in coatings or edible films, they are released slowly into the food, thereby providing a longer-lasting effect (Nazzaro *et al*., 2013).

Ziziphora is a genus of the Lamiaceae family, is a native plant in Iran and Turkey. *Z. clinopodioides* is one of the most well known aromatic and edible species within this genus. Its main phenolic compounds include pulegone (the principal compound), cineole, thymol, carvacrol, *p*-cymene, and limonene (Baygan *et al*., 2022). It has also been shown that *Z. clinopodioides* essential oil and extract exhibit greater antibacterial and antioxidant activities, respectively (Mehraban *et al*., 2007). The use of active coatings, such as chitosan (Hasan *et al*., 2019) and potato starch (Ranjbaran *et al*., 2019) containing *Z. clinopodioides* essential oil (0.5% and 1%) has increased the shelf life of chicken fillets. Incorporating *Z. clinopodioides* essential oil (1% and 2%) into polylactic acid films has also enhanced the antibacterial properties of the films (Shavisi *et al*., 2017). This study aimed to evaluate the antibacterial and antioxidant properties of alcoholic *Z. clinopodioides* extract (at 0.75% and 1.5%) alone or as active packaging in CMC-based coating on the shelf life of chicken fillets during a 12-day refrigerated storage period.

Materials and Methods

Preparation of hydroalcoholic extract of *Z. clinopodioides*

The plant was purchased from a reputable herbal store and scientifically verified before being transferred to the laboratory. The extraction was performed using a maceration method with ethanol as the solvent. Briefly, 50 g of the ground plant was combined with 600 ml of ethanol 99.8% and shaken for 72 hours at room temperature. The mixture was then filtered through Whatman No. 1 filter paper. The resulting extracts were

concentrated three times using a rotary evaporator (IKA RV-10, Germany) at 40°C (Shahbazi and Shavisi, 2015).

Preparation of coating solution

To prepare the coating solution, 20 g of CMC powder (Sigma Aldrich, USA) was added to 15 ml of oleic acid, Tween 80 as an emulsifier, and glycerol (50% w/w CMC) in 1 liter of water. The solution was heated on a stirrer for 10 minutes at 85°C to completely dissolve the CMC. After cooling, the pre-prepared *Z. clinopodioides* extract was added to the coating solutions at 0.75% and 1.5% (v/v) according to the experimental treatments and homogenized for 2 minutes using a homogenizer (Model D500, China) at 9000 rpm to ensure uniform dispersion of the extract in the coating matrix (Ghanbarzadeh and Almasi, 2011).

Preparation of experimental treatments

Chicken fillets were purchased from a reputable slaughterhouse and transported to the laboratory within 1 hour while maintaining cold chain and hygiene conditions. In the laboratory, the fillets were cut into pieces weighing approximately 60 g and washed with regular water. Based on the experimental treatments (Table. 1), they were divided into five equal groups (each group 450 ± 10 g). According to the approach by Hakim *et al*. (2018), the fillets were immersed in the coating solutions for 20 minutes. Control fillets were immersed in distilled water. After removal from the solution, the fillets were allowed to dry under a hood to form the desired coating. The fillets were then transferred to a refrigerator and stored for 12 days at 4°C, with sampling conducted every 3 days for analysis.

Table 1. Experimental treatments.

Treatments	Description
Control	Chicken fillet immersed in distilled water (without coating)
ZE 0.75%	Fillet coated with *Z. clinopodioides* (extract) 0.75% solution
ZE 1.5 %	Fillet coated with *Z. clinopodioides* (extract) 1.5% solution
CMC- ZE 0.75%	Fillet coated with carboxymethyl cellulose incorporated with an extract 0.75%
CMC- ZE 1.5%	Fillet coated with carboxymethyl cellulose incorporated with an extract 1.5%

Microbial analysis

For microbiological tests, 10 g of the chicken fillet sample was mixed with 90 ml of physiological saline and homogenized using a stomacher. The required dilutions were prepared. Samples were plated on PCA for counting mesophilic bacteria at 37°C for 48 hours and psychrophilic bacteria at 7°C for 72 hours. The results were reported as log CFU/g (Ojagh *et al*., 2009).

Chemical analysis

Initially, 30 ml of acetic acid: chloroform solution (3:2 ratio) and 0.5 ml of saturated potassium iodide solution were added to 5 g of fillet fat, which was extracted using soxhlet method. After thorough mixing, the mixture was titrated with 0.05 normal sodium thiosulfate until a light yellow color appeared. Then, 0.5 ml of 1% starch solution was added as an indicator until the blue color disappeared. The peroxide value (PV) was calculated based on the ratio of the volume of thiosulfate used to the weight of the fat sample and reported as meqO_2/Kg of meat tissue. The thiobarbituric acid (TBA) index was measured by the absorbance of the pink color produced in the reaction, using a spectrophotometer (UNICO, China). 200 ml of the homogenized chicken fillet sample was transferred to a 25-ml flask and brought to volume with 1-butanol. 5 ml of this mixture was transferred to a Falcon tube, and 5 ml of TBA reagent was added. The tubes were placed in a water bath at 95°C for 2 hours. After cooling to room temperature, the absorbance was measured at 530 nm. The results were reported as mg of malondialdehyde (MDA)/Kg of meat tissue (Egan *et al*., 1997).

To measure volatile nitrogen compounds, a kjeldahl apparatus (740-Bakhshi, Iran) was used after distilling the sample. Total volatile nitrogen (TVN) was absorbed by 2% boric acid and quantified by titration with 0.1 normal sulfuric acid. The results were calculated based on the amount of volatile nitrogen in ml/100 g of fillet (Ojagh *et al*., 2009).

Water-holding capacity (WHC) was determined based on the ratio of the weight difference of samples before and after centrifugation (Jtliangyou, China) for 15 minutes at × 1000 g to the initial weight and expressed as a percentage (Zhuang *et al*., 2008).

Statistical analysis

The data were analyzed in a two-way analysis of variance (ANOVA) using SPSS software version 20 and were expressed as mean ± standard deviation (Mean ± SD). The differences of means were compared using Duncan's multiple range test at a 95% confidence level.

Results

Microbiological changes

The results regarding the effect of *Z. clinopodioides* extract alone or in combination with CMC-based coating on the mean counts of mesophilic and psychrophilic bacteria were shown in Fig. 1. Over time, the mean counts of mesophilic and psychrophilic bacteria increased in all groups ($p < 0.05$). The application of *Z. clinopodioides* extract, especially when incorporated into the CMC-based coating, significantly slowed this trend. The antibacterial effect of the extract was also concentration-dependent. At the end of the storage period, the lowest mean counts (log CFU/g) of mesophilic (5.00 ± 0.55) and psychrophilic (3.90 ± 0.20) bacteria were observed in fillets with active coating containing 1.5% extract.

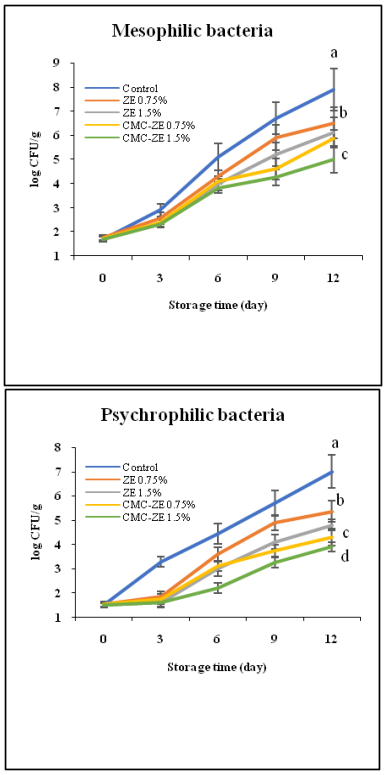

Fig. 1. Effect of experimental treatments on mean count of mesophilic and psychrophilic bacteria (log CFU/g) in chicken fillets during refrigerated storage. ZE: *Z. clinopodioides* extract; CMC: Carboxymethyl cellulose. Values (n=3) were expressed as mean ± standard deviation ($p < 0.05$)

Total volatile basic nitrogen

As shown in Fig. 2, the mean of this indicator significantly increased over time. Meanwhile, the active packaging containing 1.5% extract clearly prevented the acceleration of this increase from day 9 of storage, and at the end of the storage period, the fillets with this packaging had significantly lower TVB-N values (16.45 ± 1.4 mg/100 g) compared to other fillets (26.70 ± 1.80 mg/100 g in the control).

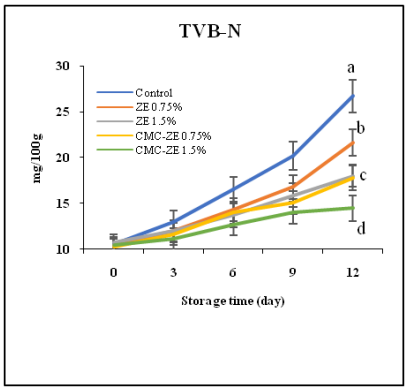

Fig. 2. Effect of experimental treatments on mean of total volatile basic nitrogen (mg/100 g) in chicken fillets during refrigerated storage. ZE: *Z. clinopodioides* extract; CMC: Carboxymethyl cellulose. Values (n=3) were expressed as mean ± standard deviation ($p < 0.05$)

Antioxidant capacity assay parameters

As illustrated in Fig. 3, the PV significantly followed an increasing trend in all groups until the last day of storage ($p < 0.05$). However, at day 12, the PV of fillet coated with CMC-based containing 1.5% extract was 57.8% lower than the control group (7.00 ± 0.45 vs 2.95 ± 0.57 meq/Kg). Similar to the PV rate, the TBA values were also enhanced during storage. The differences between the higher and lower values of TBA, observed in the control and CMC-extract 1.5%, were 48.43% (0.95 ± 0.11 vs 0.65 ± 0.07 mg MDA/Kg).

Z. clinopodioides extract concentration significantly affected antioxidant capacity parameters. By increasing the extract concentration from 0.75% to 1.5%, the PV and the TBA value decreased by 28.7% and 15%

respectively.

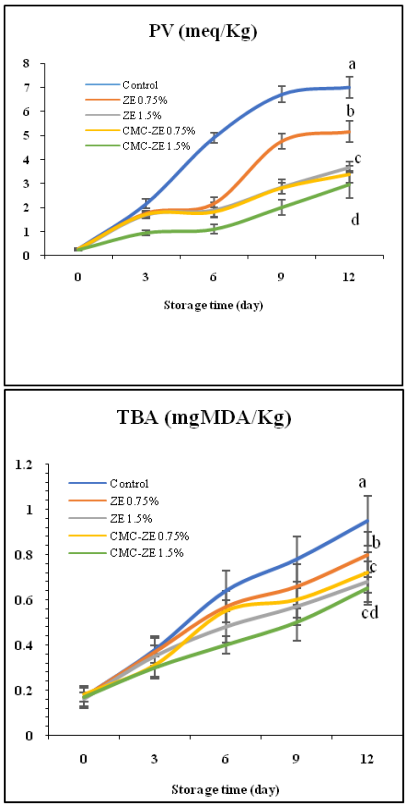

Fig. 3. Effect of experimental treatments on mean of peroxide value (PV; meq/Kg) and thiobarbituric acid (TBA) index (mg MDA/Kg) in chicken fillets during refrigerated storage. ZE: *Z. clinopodioides* extract; CMC: Carboxymethyl cellulose. Values (n=3) were expressed as mean ± standard deviation ($p < 0.05$)

Water holding capacity

As presented in Table 2, the WHC of fillets decreased during storage. However, the CMC coating prevented the rapid and sudden drop in WHC. While the drop in WHC in the uncoated fillets was more than 36%, this value was about 21-21.5% in the coated fillets. In fact, it can be resulted that the CMC-based coating was able to prevent about 15% of the drop in WHC in the fillets.

Table 2. Effect of experimental treatments on mean of water holding capacity (%) in chicken fillets during refrigerated storage.

Treatments	Refrigerated storage time (day)				
	0	3	6	9	12
Control	67.00±4.32^{a}	56.75±4.00^{b}	50.59±3.60^{b}	44.00±3.00^{b}	42.17±2.00^{b}
ZE 0.75%	67.50±3.62^{a}	57.00±4.02^{b}	51.15±3.00^{b}	45.70±3.00^{b}	43.00±2.10^{b}
ZE 1.5%	68.00±4.02^{a}	56.90±3.72^{b}	51.00±3.05^{b}	45.00±2.20^{b}	42.95±2.30^{b}
CMC-ZE 0.75%	67.00±4.00^{a}	62.75±3.65^{a}	59.10±3.10^{a}	56.60±2.40^{a}	52.95±3.00^{a}
CMC-ZE 1.5%	67.70±3.15^{a}	63.15±3.22^{a}	59.75±3.00^{a}	56.70±2.67^{a}	53.15±3.05^{a}

Discussion

Chicken meat, due to its nutrient content and moisture, is prone to spoilage, especially when stored under inappropriate conditions. Microbial and chemical spoilage of chicken not only contributes to food poisoning but also incurs substantial economic losses. According to Iranian national standards (No. 9714 regarding fresh chicken meat), the maximum bacterial count allowable in meat is set at 7 log CFU/g. While the mean counts of mesophilic and psychrophilic bacteria in the control group exceeded the allowable limit at the end of the storage period, none of the treated groups with the extract exceeded the established limits. Probably due to the protective effect of the edible CMC coating, the antimicrobial effect of the *Z. clinopodioides* extract due to phenolic compounds, and the synergistic effect of the coating and the extract, the bacterial count in the treated chicken fillets was lower than in the control (Shavisi *et al*., 2017; Mehdizadeh *et al*., 2018). Given that the antibacterial properties of monoterpenoids present in *Z. clinopodioides* have been widely studied and proven, it is not surprising that extracts containing high amounts of these compounds exhibit potent antibacterial activity. It has been shown that their antibacterial effect is based on targeting the cytoplasmic membrane, leading to bacterial lysis and leakage of intracellular contents (Mazarei *et al*., 2023).

Total volatile basic nitrogen (TVB-N) is one of the main quality assessment indicators for meat and a key marker of meat spoilage. In general, the use of the extract in a concentration-dependent manner, especially when incorporated into the CMC matrix, resulted in a decrease in TVB-N over storage. The lower level of TVB-N in the treatment containing the extract compared to the other treatments can be attributed to the reduction in the bacterial population of the aforementioned treatments or the reduction in the oxidative ability of the bacteria to separate amines from non-volatile nitrogen compounds, or both factors as a result of the effect of the extract on the bacteria present in the fillet. The activity of proteolytic bacteria and enzymatic activity is the reason for the increase in the production of volatile basic compounds. *Z. clinopodioides* extract prevents the

breakdown of proteins by inhibiting spoilage bacteria, due to its antimicrobial compounds, thereby preventing the release of nitrogen compounds (Burt, 2004). These results are consistent with the results of the antibacterial effect evaluation of the extract (Fig. 1) and are in accordance with the results of similar studies (Hasan *et al*., 2019; Rajabian *et al*., 2019).

According to the instructions of the Office of Health Supervision, Evaluation and Risk Management of Domestically Produced Poultry Meat (No. 01.400), if the amount of TVB-N in meat exceeds 27 mg/100 g of meat, the meat is inedible, and if it is a maximum of 20 mg/100 g of meat, it is acceptable. Meat with TVB-N within the mentioned values is edible and should be used as soon as possible (Iran Veterinary Organization, 2021). Accordingly, none of the fillets fell into the unusable range during storage. However, at the end of the storage period, fillets coated with a 0.75% extract were in the range (21.67 ± 1.44 mg/100 g) that should be used immediately. However, using the extract at a higher concentration or in active packaging resulted in fillets falling into the desired range (less than 20 mg/100 g) even after 12 days of storage in the refrigerator.

Peroxide compounds are odorless and tasteless substances formed during the early stages of fat oxidation, evaluated by measuring the PV. The increased PV could be due to the higher rate of hydroperoxide formation than its decomposition to secondary metabolites during storage (Ibrahim Sallam, 2007). Higher extract concentration (1.5%) had a clearly greater antioxidant effect. In addition, the extract in active packaging CMC-based was significantly more effective. This result confirmed the slow and continuous release of the extract in the CMC biopolymer, which led to its greater effectiveness.

Phenolic and flavonoid compounds of plants can act as electron donors within the cells and show reducing and antioxidant properties (Sakihama *et al*., 2002). The high content of phenols and flavonoids in the *Z. clinopodioides* can explain its antioxidant activity. Active packaging is known as an effective factor in preventing oxygen penetration. The edible coating acts as a barrier between the fillet and the surrounding environment and reduces the penetration of ambient oxygen through the surface into the fillet (Khademi Shurmasti *et al*., 2021). The CMC coating significantly reduced the peroxide value in treated fillets compared to the control samples. Higher concentrations of extract had a significant impact on its effectiveness and significantly improved the efficiency of the coating.

Fat oxidation is related to the oxidation of polyunsaturated fatty acids, which leads to the development of undesirable odors and flavors in food products and ultimately shortens their shelf life. Thiobarbituric acid is used as an indicator of the degree of secondary oxidation of fat and is due to the presence of TBA- reactive substances (TBARS) resulting from the second stage of spontaneous oxidation, during which peroxides are oxidized to substances, such as aldehydes and ketones (Ibrahim Sallam, 2007). The high hydrogen-donating capacity, free radical absorption and significant antioxidant activity of *Z. clinopodioides* monoterpenes, including polgon, cineole, thymol, carvacrol, p-cymene and limonene, have been proven. Compounds with antioxidant activity in *Z. clinopodioides* extract prevent the continuation of the oxidation process and the increase of TBRS by reducing the oxidation products (Baygan *et al*., 2022).

It was expected that the phenolic compounds in the *Z. clinopodioides* extract would delay the oxidation process of fats and prevent their conversion into primary (hydroperoxide) and secondary (aldehyde and ketone) products. It was also clearly seen that the CMC matrix caused a slow and continuous release of the extract to the fillets and the protective role of the extract against oxidation was maintained until the final day of storage. In similar studies, the use of various polysaccharide matrices and phytochemical compounds delayed the increasing trend of this index and preserved the quality and shelf life of the meat, as mentioned in introduction.

One of the criteria for evaluating meat quality is the measurement of the WHC of meat. The use of active packaging significantly slowed down the decline in WHC, and the concentration of the extract had no significant effect on this. The polyphenolic compounds in the *Z. clinopodioides* extract, by controlling the oxidation reactions in the meat, maintain the water storage space between myofibrils and increase the water retention capacity, because the oxidation of fats and proteins and all factors that change the state of myofibril proteins are effective in the rate of moisture loss of meat. The degree of protein

denaturation is also an important factor in the rate of water loss and the reduction in WHC (Leygonie *et al*., 2012). Although the effect of extract concentrations on the WHC of fillets was not statistically significant, considering the favorable effects that the chemical compounds of the extract alone or in active packaging had on the microbial spoilage and oxidative spoilage indices of fillets, higher WHC was expected in fillets containing extract, especially in active packaging. Consistent with the results of this study, the use of active composite packaging containing plant extract increased the storage capacity of chicken fillets (Mardani Kiasari and Khademi Shurmasti, 2020).

In this study, it was shown that *Z. clinopodioides* extract, with its phenolic compounds, was able to exhibit its antimicrobial and antioxidant effects in a concentration-dependent manner. On the other hand, CMC-based active packaging containing *Z. clinopodioides* extract improved the efficiency of the extract, and the microbial spoilage and oxidative spoilage indices of fillets packaged with it had conditions that are more favorable. According to the indicators studied the use of active biodegradable packaging of CMC-*Z. clinopodioides* extract (1.5%) is recommended to increase the shelf life of chicken meat for at least 6 days at refrigerator temperature. Of course, sensory evaluation of the product will lead to better decision-making regarding the desired concentration.

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Conflict of Interest

There is no conflict of interest among the authors.

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