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Extended Shelf Life of Chicken Meat Using Carboxymethyl Cellulose Coated Polypropylene Films Containing *Zataria multiflora* Essential Oil

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Abstract**—**The purpose of the present study was to evaluate carboxymethyl cellulose (CMC) coated polypropylene (PP) films containing *Zataria multiflora* (ZEO) essential oils (4%) as an antimicrobial packaging for chicken breast stored at 4 °C. To increase PP film hydrophilicity, it was treated by atmospheric cold plasma prior to coating by CMC. Then, different films including PP, PP/CMC, PP/CMC containing 4% of ZEO were used for the chicken meat packaging in vapor phase. Total viable count and pseudomonads population and oxidative (TBA) changes of the chicken breast were analyzed during shelf life. Results showed that the shelf life of chicken meat kept in films containing ZEO improved from three to nine days compared to the control sample without any direct contact with the film. Study of oxygen barrier properties of bilayer film without essential oils (0.096 cm^3 μm/m^2 d kPa) in comparison with PP film (416 cm^3 μm/m^2 d kPa) shows that coating of PP with CMC significantly reduces oxygen permeation of the obtained packaging ($P<0.05$), which reduced aerobic bacteria growth. Chemical composition of ZEO was also evaluated by gas chromatography–mass spectrometry (GC–MS), and this shows that thymol was the main antimicrobial and antioxidant component of the essential oil. The results revealed that PP/CMC containing ZEO has good potential for application as active food packaging in indirect contact which would also improve sensory properties of product.

Keywords**—**Shelf life, chicken breast, polypropylene, carboxymethyl cellulose, essential oil.

I. Introduction

HIGH nutritional value and low-cost production of poultry meat have caused them to be among the most popular food products. However, spoilage of poultry meat is an economic obstacle to the industry which can also result in public health hazard since poultry meat can be subjected to the growth of both spoilage and pathogenic microorganisms. Because poultry meat belongs to perishable foods, their shelf-life extension is the main worry of industries for these products [1].

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The problem is that some of meat preserving methods are expensive, not scalable and have undesirable effects on food. One common strategy to preserve raw or minimally-processed fresh foods such as meat is the use of natural preservatives. Essential oils from ZEO plant have been approved by FDA as safe additives. This essential oil has proven medicinal, antioxidant, and antimicrobial properties that have been widely discussed in the literature [2] which is attributed to their high content of phenolic compounds. Although use of many essential oils as GRAS food preservatives has no safety limit, their strong flavor is an important limitation for their overuse in food products since effective antimicrobial levels may overpass the least organoleptic acceptance levels [3]. On the other hand, direct addition of natural or chemical preservatives to the meat is challengeable because the protective effect of antimicrobials is limited following the interactions in the complicated food system [4].

One of the most novel strategies to overcome these restrictions is antimicrobial packaging. This packaging helps the release of additives at a controllable rate; therefore the critical concentration needed for microbial inhibition on surface is maintained during the food storage [5]. This packaging could directly release active material to the food stuff, or indirectly release it to the headspace; however, the latter is far better in case where there is a concern about unacceptable organoleptic changes.

Biopolymer based films as an alternative to synthetic polymers has generated much interest in food industry due to their environmental issues [6]. CMC is recognized as a GRAS polymer and is prevalently used in food and have good film forming ability [7]. However, bio-based polymeric film has weak mechanical properties. Therefore, production of a bilayer film which composed of a synthetic layer and a degradable layer such as CMC, can improve poor mechanical and water vapor barrier properties of biopolymer-based films. PP, as a versatile polyolefin polymer, is widely used in food packaging industry because of its low cost, heat resistance, and good processability, but it is known to have relatively low oxygen barrier properties/resistance, which is an important factor in food packaging during storage [3]. Coating of PP film with CMC as a new composite structure can improve thermal, mechanical, and barrier properties of CMC film. Furthermore, CMC layer can act as a carrier of antimicrobial agents such as essential oils in the packaging.

For good adhesion of non-polar PP layer to polar CMC film and production bilayer films, it is necessary to make surface modification of the PP, which can be performed by cold plasma. Cold plasma enhances PP surface energy levels prior to coating with a second layer [8], [9].

The purpose of the present study was to evaluate efficacy of active composite bilayer films based on CMC/PP containing ZEO on chicken meat preservation at 4 °C in indirect contact to the product (vapor phase).

II. Methods and Materials

A. Materials

Fresh chicken meat in the first production day was provided by a local shop (Tehran, Iran). Chicken samples weighing about 50 g were placed in polystyrene dishes. Commercial CMC was supplied by Sinocmc Co., LTD (Qingdao, China). PP films with 20 micrometer thickness were purchased from Pol-film Co. (Tehran, Iran). Essential oil was catered by Barij Essence Pharmaceutical Co. (Kashan, Iran). Tween 80, glycerol, thiobarbituric acid, boric acid, methyl red and sulfuric acid were bought from Merck Co. (Darmstadt, Germany). 1- butanol solvent was purchased from Samchun Co. (Korea). Microbiological media including Plate Count Agar were supplied by Liofilchem Co. (Teramo, Italy). All other reagents used were of analytical grade.

B. Identification of Essential Oils' Components

Identification of components was performed using GC–MS. For detection and quantification of compounds in the essential oils, 20 µl of ZEO was diluted with 1 ml cyclohexane, and approximately, 2 µL of the sample was injected. The constituents of the oils were identified by comparison of their mass spectra with those of a computer library or with standard compounds and approved by comparison of their retention times, with those of authentic compounds [10].

C. Surface Modification of PP Films

PP film was cut into rectangles of 20*30 cm, and the stone-washed film treated by high-voltage atmospheric plasma treatment (roll to roll plasma system, Satiya Co. Iran) for 10 seconds to improve the attachment of PP surface to the CMC solution in the coating process. The plasma treatment applied was a roll to roll plasma system with 30*30*110 cm dimensions, roller length of 80 cm, and the gap distance of 2 mm. Each roller with four electrodes made of stainless steel had a power of about 3 kW.

D. Film Preparation

The bilayer films were made by the casting method. CMC solution (1%, w/v) was prepared by dissolving CMC powder in distilled water containing glycerol (50% (w/w)) at 75-80 °C for about 40 min with continuous stirring. The film-forming dispersion (FFD) was cooled to about 55 °C to eliminate any air bubble (control film). ZEO was added to the FFD at a concentration of 4% following the addition of tween 80 as an emulsifier (0.4% v/v). All the solutions were blended for about 4 min using a laboratory mixer (Atomix, Switzerland), and then were homogenized by rotor-stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) at 13,500 rpm for 4 min at 70 °C, and were finally degassed by cooling FFDs to 55 °C. The FFDs were casted on the center of rectangular plastic plates which were previously covered with plasma treated PP films, and were dried at 35 °C for about 20h.

E. Film Parameter

1. Film Thickness

For determination of film thickness, a manual micrometer (Mituto, Tokyo, Japan) with the sensitivity of 0.001 mm was applied. Values were reported as an average of at least ten random spots for each film.

2. Oxygen Permeation

Oxygen permeability (OP) of films was measured at 23-25°C and 50% RH using an Oxygen Permeation Analyzer (Brugger, Germany) according to the American Society for Testing Materials (ASTM) Standard method D 3985-81 (ASTM, 1980) [11]. A film was placed on the sample holder with an exposed testing area of 78.4 cm^2. Nitrogen gas flew on one side and pure oxygen gas flew on the other at the above condition. The coated side of the bilayer films (CMC) was exposed to flowing nitrogen gas and the opposite side (PP) to flowing oxygen gas.

F. Shelf Life Studies

1. Chemical Analysis

The pH of chicken meat was measured periodically using a digital pH meter (Multiline P4WTW). 10 g of chicken samples was thoroughly homogenized with 100 ml of distilled water, and the homogenate was used for pH determination. TBA was determined according to the method proposed by Ojagh et al. [12]. Thiobarbituric acid content was expressed as milligrams of malonaldehyde (MA)/ kg chicken.

2. Microbiological Analysis

25 g of the chicken meat sample was aseptically cut and blended with 225 ml of sterile physiological saline (0.9% salt solution) for about 2 min. For microbial enumeration, appropriate serial decimal dilutions were prepared by mixing a 1-ml sample with 9 ml of sterile physiological saline. Total viable counts (TVC) were determined by pour plate method using sterile molten (45 °C) Plate Count Agar (PCA, Liofilchem, Teramo, Italy) after incubation for 2 days at 37 °C. Then, 0.1 ml of the serial dilutions of chicken homogenates was spread on the surface of dry media. All plates were examined visually for typical colony types and for morphology characteristics related to each growth medium.

3. Sensory Evaluation

Small cuts of the chicken meat samples were steamed for about 1.5h. A group of 30 untrained panelists was used for sensory evaluation of chicken meats kept in different packaging at 4 °C. 5-scaled Hedonic test was used for sensory evaluation of samples. Panelists were asked to evaluate taste, odor, color, texture, and total acceptance of the cooked samples. The scale points were: excellent, 5; very good, 4; good, 3; acceptable, 2; poor, 1.

G. Statistical Analysis

The statistical analysis of the data was performed using SPSS statistical software version 21 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) followed by the Duncan's multiple range test was used to determine any significant differences among the treatments at a 95% confidence level. In the shelf life test, repeated measure design (RMD) was used for comparison of average responses at different days. Significant difference in sensory evaluation of chicken meat samples was determined through Kruskal-Wallis test.

III. Results and Discussion

Table I shows different treatments used for chicken meat packaging.

TABLE I
PACKAGING USED FOR CHICKEN MEAT SAMPLES

Type of packaging	Treatment number
PP	N_1
PP/CMC	N_2
PP/CMC incorporated with 4% ZEO	N_4

A. Identification and Quantification of Volatile Compounds

In general, 5-6 components were identified in ZEO essential oil representing 78.7% the oil compositions (Table II). The major constituents of ZEO essential oil were thymol, carvacrol and p-cymene comprising 48.2%, 13.8%, and 8.5% of the total composition, respectively.

In contrast to our study, Ebrahimzadeh et al. identified less carvacrol (2.35%) and more γ-terpinene (21.5%) in ZEO essential oil [13]. Regarding the same extraction method used in the present work and the mentioned reports (steam distillation), variation in the chemical composition of the essential oils may be attributed to the type of the plants (different plant sources and chemotypes, different parts of the plant, growth stages), variation in seasons, different environmental, cultivation, or storage conditions [14].

TABLE II
DIFFERENT COMPONENTS IDENTIFIED IN ZEO

Number	Type	Retention time (min)	Concentration (%)
1	α-pinene	10.313	1.4
2	camphene	10.814	-
3	β-pinene	11.795	-
4	β-myrcene	12.344	0.8
5	β-phellandrene	12.802	-
6	4- carene	13.197	-
7	p-cymene	13.551	8.5
8	γ-terpinene	14.644	6
9	α-terpineol	19.119	-
10	thymol	22.572	48.2
11	carvacrol	22.845	13.8
12	caryophyllene	25.178	-
Total			**78.7**

B. Film Thickness

Thickness of the films used for chicken meat packaging varied from 0.019 to 0.125 mm. PP film had the lowest thickness; however, coating of CMC led to an increase to 0.048 mm representing a significant difference in the film thickness ($P<0.05$). Furthermore, incorporation of ZEO led to an increase in the film thickness to 0.120 compared to PP/CMC film, ($P<0.05$). This can be attributed to the entrapment of essential oil microdroplets into the film structure.

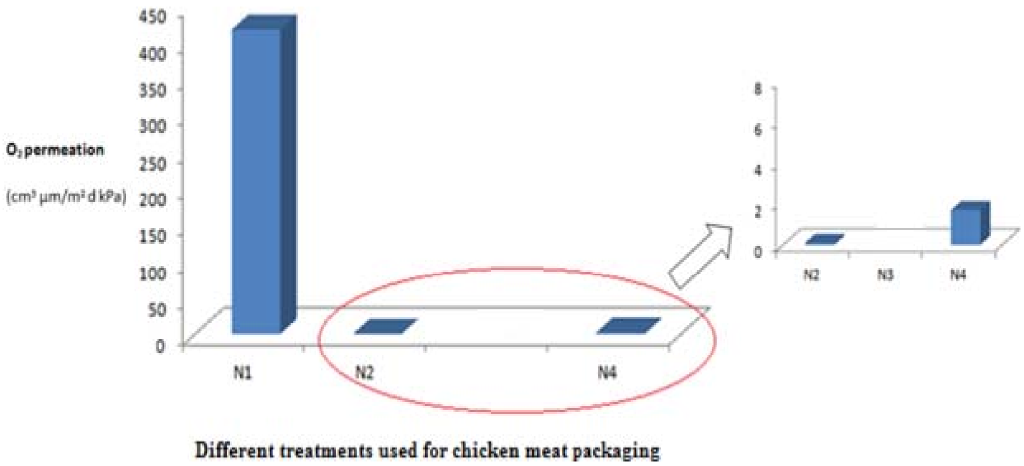

Fig. 1 Oxygen permeability of different films used for chicken meat packaging

C. Oxygen Permeability

One of the most important characteristics of food packaging materials is gas permeability. Barrier to oxygen transmission is a key factor for food preservation [15]. Fig. 1 illustrates oxygen permeation of film samples used for chicken meat packaging. PP film had relatively high permeability to oxygen (416 cm^3 μm/m^2 d kPa) at 25 °C, which is due to its polyolefin nature. Coating PP films with CMC layer could considerably reduce O_2 permeation to 0.096 cm^3 μm/m^2 d kPa. Biopolymer based films are considered good oxygen barriers because of their hydrophilic nature acting against diffusion of non-polar compounds through the film matrices (15) and due to their firmly packed structure made of hydrogen bonds [16].

Incorporation of ZEO increased O_2 permeation of films compared to PP/CMC bilayer film. However, it was still much lower than PP film. Oxygen permeability trough the film matrix is affected by solubility and diffusion of its molecules. Higher solubility of oxygen can be expected in non-polar EO phase which resulted in more oxygen transfer (17).

D.Shelf Life Study

1. Chemical Changes

Increase in pH discloses extent of meat spoilage due to protein decomposition which leads to the formation of free amino acids, and subsequently the production of NH_3 and amines [17]. The initial pH of fresh chicken meat was about 5.7 which increased during period of storage at 4 °C in all treatments (Fig. 2). However, this increase was not statistically significant in the first 3 days ($P > 0.05$). PP/CMC film containing 4% ZEO resulted in a slightly lower pH (5.8) in the chicken meat on day 3 compared to the other treatments, which implies the partial protective effect of ZEO against protein breakdown.

The initial TBA content in fresh chicken meat was low indicating low degree of lipid oxidation (Fig. 3). During storage in 4 °C, TBA content of all chicken meat samples increased; however, this increase was meaningfully higher in N_1 compared to other treatments ($P<0.05$), which can be due to high oxygen permeability of PP film. TBA values of chicken treatments were lowest in sample N_4 after 3 days of storage. Lower values of TBA in N_4 samples are probably related to antioxidant activity of ZEO resulting from the presence of phenolic compounds such as carvacrol, thymol, p-cymene and ɣ-terpinene. Result of this study is in agreement with the finding of Radha Krishnan et al. who reported that adding phenolic-rich extracts protects chicken meat against lipid oxidation [18].

2. Microbiological Changes

Table III shows enumeration of microbial population including total viable counts in packed chicken meat samples stored at 4 °C. The initial TVC of chicken meat was ca. 5.44 log cfu/g (day 0). After three days of storage, the chicken samples kept in the PP (N_1) and PP/CMC (N_2) films showed a significant increase ($P < 0.05$) in TVC and reached to 9.09 and 6.94 log cfu/g, respectively. This indicates that both chicken samples had exceeded the acceptable level ($5*10^6$ log cfu/g according to food and drug organization), and were spoiled. TVC in N_2 sample was significantly lower than N_1 which is due to better inhibitory effect of bilayer film against oxygen transmission. On the contrary, sample packaged in ZEO containing films (N_4) showed a meaningful decrease ($P < 0.05$), and reached to 5.2 log cfu/g. Besides low oxygen permeability of emulsified films, this reduction could be related to phenolic compounds of essential oil which have proved antioxidant activities.

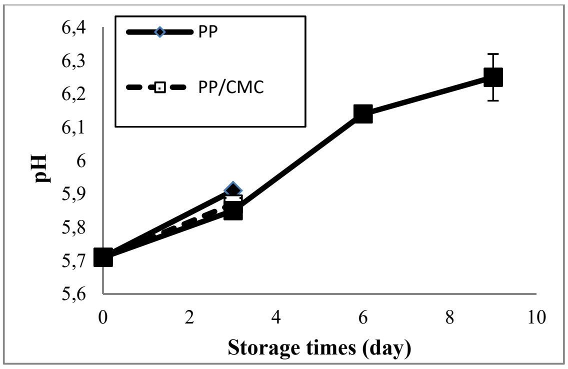

Fig. 2 Mean pH values of raw chicken meat stored in different packaging at 4 °C

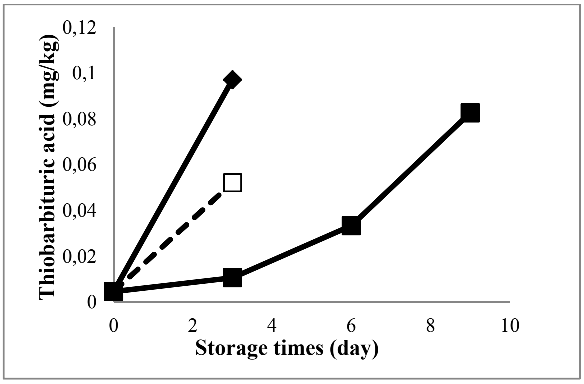

Fig. 3 TBA values (mg of malonaldehyde/kg of meat) of raw chicken meat stored in different packaging at 4 °C

TABLE III
ENUMERATION OF TOTAL VIABLE COUNT (TVC) IN CHICKEN MEAT STORED AT 4°C

Treatments	Log CFU/g			
	day 0	day 3	day 6	day 9
Total viable count (TVC)	$5.45{\pm}0.05^{A}$	-	-	-
N_1	$5.45{\pm}0.05^{A}$	$9.10{\pm}0.01^{aB}$	-	-
N_2	$5.45{\pm}0.05^{A}$	$6.95{\pm}0.02^{bB}$	-	-
N_4	$5.45{\pm}0.05^{A}$	$5.20{\pm}0.02^{dB}$	$5.73{\pm}0.01^{bA}$	$7.17{\pm}0.08^{C}$

$^{a-d}$ Values within a column followed by a different small letter are significantly different from each other on a specific day ($P<0.05$) according to Duncan test.
$^{A-D}$ Values within a row followed by a different capital letter are significantly different from each other regarding each treatment ($P<0.05$) according to LSD test.

TABLE IV
SENSORY ATTRIBUTES OF DIFFERENT CHICKEN MEAT TREATMENTS

Treatment	Taste	Odor	Color	Texture	Total acceptance
N_1	2.29±0.91^b	2.36±1.08^b	4.5±0.65^a	3.86±0.77^b	2.71±0.82^b
N_2	2.71±0.82^b	2.79±0.89^b	4.5±0.65^a	4.07±0.73^{ab}	3.07±0.73^b
N_4	3.86±1.09^a	3.86±1.23^a	4.36±0.74^a	4.57±0.51^a	4.14±0.77^a

Values within a column followed by a different small letter are significantly different from each other (P<0.05) according to Mann Whitney test.

3. Sensory Evaluation

Table IV shows the results of sensory evaluation of chicken meats kept in different packaging. Use of antimicrobial PP/CMC films for packaging had a significant effect on chicken meat sensory perception. Panelists gave higher scores to N_4 sample indicating that they were highly preferred as judged by taste, odor, and total acceptance in comparison to N_1 and N_2 samples ($P < 0.05$). This implies that incorporation of ZEO in the packaging improved sensory properties of chicken meat except for color that was similarly scored in all treatments. No significant difference was recognized between chicken meat kept under PP or PP/CMC films (N_1 and N_2, respectively) for all sensory attributes ($P > 0.05$).

Application of active films in the gas phase in which the active compounds are released to the headspace, seems to improve the sensory properties of the food products as the results of sensory evaluation corroborated this claim (Table IV).

IV. CONCLUSION

Application of active bilayer films for chicken meat packaging as a novel conservation method was investigated in the gas phase. CMC coated PP films containing 4% ZEO essential oils could effectively increase the shelf life of chicken meat to 9 days by retarding microbial and oxidative spoilage. Furthermore, sensory evaluation of chicken meats interestingly showed that those stored in the antimicrobial packaging were more preferable considering taste, odor, and total acceptance. Therefore, ZEO incorporated PP/CMC films can be potentially used to extend shelf-life and improve sensory properties of chicken meat in the gas phase.

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