



Chicken Hamburger Preservation Using Antimicrobial Packaging Containing Cinnamon Extract

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Original article</p>	<p>Introduction: Raw meat is recognized as one of the most vulnerable foodstuffs. The present study aimed to assess the preservation of raw chicken hamburger coated with the blends of chitosan (CH) and gelatin (GE), activated with 1% cinnamon extract (CE) and stored in refrigerated conditions for 10 days.</p> <p>Methods: The chicken hamburger samples (10 g) were aseptically cut and mixed in a stomacher with 90 milliliters of sterile buffered peptone water for two minutes. The proper dilution was inoculated onto microbiological plates, including Baird-Parker agar (<i>Staphylococcus aureus</i>), violet red bile glucose (VRBG) agar (total coliforms), and plate count agar (aerobic plate count (APC)). Peroxide value (PV) analysis was also performed based on standard method.</p> <p>Results: On sampling days zero, two, four, six, eight and ten, all the samples coated with edible films (especially those enriched with 1% CE) had excellent effectiveness against lipid oxidation compared to uncoated samples. After 10 days of storage, all the films could effectively decrease the growth of <i>Staphylococcus aureus</i>, total coliforms, and aerobic plate count (APC) in chicken hamburgers compared to controls ($P < 0.05$).</p> <p>Conclusion: It could be concluded that edible bioactive packaging films containing CH- and GE-based polymers incorporated with 1% CE could be a proper alternative for improving chicken hamburger preservation.</p>
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Introduction

Raw meat is recognized as one of the most vulnerable foodstuffs (1). Fresh chicken meat has an extremely short shelf life (2-5 days) when preserved at refrigerated temperature ($4 \pm 1^\circ\text{C}$) depending on the type of packaging (e.g., plastic, antimicrobial, modified atmosphere, and vacuum packaging) (2). This has been associated with the high water content, pH, and chemical composition of chicken meat, which are appropriate for the growth of microbial spoilage and pathogens to the permitted microbiological limits, thereby remarkably contributing to meat spoilage (3, 4). On the other hand, oxygen adversely affects the shelf life attributes of various fresh foodstuffs. After microbial spoilage, lipid oxidation is considered to be the most important cause of food decomposition (5).

Design of the biodegradable films and coatings containing active, natural agents for the preservation of foods represents a novel method for improving the quality attributes of raw and fresh foodstuffs (6). In this regard, gelatin (GE) has been used in biodegradable film production owing to its edible and biodegradable nature, compatibility with polymers, cost-efficiency, and wide availability of the sources (7, 8). In addition, chitosan (CH) has received significant attention considering its biodegradability, edibility, biocompatibility, film form ability, low cytotoxicity, and intrinsic antimicrobial activity, which have been demonstrated in numerous foods, such as meat products. CH has been approved by the United States Food and Drug Administration (FDA) as a "Generally

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Recognized as Safe (GRAS)" food additive (8).

The demand for natural additive compounds (e.g., essential oils and natural extracts) has increased in foodstuffs, representing an alternative to synthetic additives (9). *Cinnamomum zeylanicum*, also known as cinnamon, contains cinnamaldehyde, beta-caryophyllene, linalool, and other terpenes. Cinnamaldehyde is the most abundant compound of cinnamon leaf essential oil, which causes the special odor and flavor of cinnamon (10). It is frequently applied as a food preservative and flavoring agent, and the FDA has listed it as a GRAS (11).

Several studies have been focused on the antimicrobial effects of natural extracts and essential oils of various plants, such as oregano (12, 13), thyme (14), mint (15), olive leaf (16), and sage (13) in foods, confirming that these extracts could decrease the growth of microbial spoilage and pathogens, delay lipid and protein oxidation, improve sensory attributes, and increase the shelf life of fresh food products.

The present study aimed to assess the preservation of chicken hamburger -coated with the blends of CH and GE, activated with 1% cinnamon extract (CE) and stored in refrigerated conditions for 10 days.

Material and methods

Experimental Materials

CH and GE powders with medium molecular weight were supplied by Sigma-Aldrich (United Kingdom), and CE was obtained from Gol Adonis Daru Company (Tehran, Iran). All the chemicals and culture media were purchased from Sigma-Aldrich and Merck companies (Germany), respectively.

CH and GE Film Preparation

CH and GE solutions were prepared separately. One gram of CH was dissolved in 100 milliliters of 1% v/w glacial acetic acid. In parallel, four grams of GE was dissolved in 100 milliliters of distilled water, hydrated for seven minutes, and solubilized in a thermostatic bath at the temperature of 55 °C. Afterwards, glycerol (0.75%) was added to the solutions. The studied film-forming solutions included the films based on pure CH, blended films (CH: GE=50:50), and similar films based on 1% CE. For the films containing 1% CE, one gram of the extract was added to the designated film-forming solutions

and continuously stirred on a hot plate magnetic stirrer (IKA, UK) at the temperature of 24±1°C for 30 minutes. The active films were prepared using a casting technique and air-dried at room temperature. Following that, the films were cut off and conditioned at room temperature and 53% relative humidity in chambers for 48 hours (7, 17).

Chicken Hamburger Preparation

Chicken thigh meat was obtained from a poultry slaughterhouse in Kermanshah, Iran and processed after four hours of slaughter deboning in order to produce chicken hamburger samples (diameter: 6 cm, thickness: 0.5 cm). Both surfaces of the hamburger samples were packed with the prepared films. Two non-coated (without film) and coated samples were placed in foam trays, wrapped with a commercial stretch plastic film, and stored for up to 10 days.

Chicken Hamburger Analysis during Refrigerated Storage

In total, 30 grams of the chicken hamburger samples were mixed in 210 milliliters of chloroform: methanol: distilled water (60:120:30) using a rotor-stator homogenizer at 10,000 rpm for one minute at refrigerated temperature. The homogenate was diluted with 60 milliliters of chloroform and homogenized at 10,000 rpm for 30 seconds. Afterwards, 60 milliliters of distilled water was incorporated, and the mixture was homogenized again for 30 seconds. The homogenate was centrifuged at 4,500 rpm for 10 minutes at refrigerated temperature, and the supernatant was transferred to a separating flask. The chloroform phase was drained off into a 250-milliliter Erlenmeyer flask containing 2-5 grams of anhydrous sodium sulfate. The mixture was stirred well and filtered into a round-bottom flask through Whatman Grade 4 filter paper.

Finally, the solvent was evaporated through drying in a sterilization oven at the temperature of 100°C. The extracted lipid was subjected to peroxide value (PV) analysis. The chicken hamburger lipid (3 g) was dissolved in a 2:3 v/v mixture of chloroform and acetic acid. Following that, an oversaturated potassium iodide solution (0.5 ml) was added. The mixture was stirred for one minute, followed

by the addition of 30 milliliters of distilled water. In the next stage, titration with sodium thiosulfate solution (0.001 N) was performed using starch as an indicator. PV was expressed as the milliequivalents of the peroxide index per kilogram of the lipid (18). All the analyses were performed in triplicate.

Microbiological Analysis

The chicken hamburger samples (10 g) were aseptically cut and mixed in a stomacher with 90 milliliters of sterile buffered peptone water for two minutes. In addition, aliquots were serially diluted in buffered peptone water. Following that, 0.1 milliliter of each dilution was inoculated onto microbiological plates, including Baird-Parker agar (*Staphylococcus aureus*), violet red bile glucose (VRBG) agar (total coliforms), and plate count agar (aerobic plate count (APC)). The plates were incubated at the temperature of 37°C, and the colonies were analyzed after 24 hours for *S. aureus* and total coliforms and after 48 hours for aerobic microorganisms (19). All the analyses were performed in triplicate.

Statistical Analysis

Designated experiments and microbial and chemical analysis were performed in triplicate. The microbiological data were expressed as log CFU/g. Data were investigated in terms of normality and variances for homogeneity using

Kolmogorov-Smirnov test and Levene's test, respectively. Moreover, significant differences were determined at the P-value of less than 0.05 using the analysis of variance (ANOVA) and Duncan's multiple range test in SPSS version 16.

Results and Discussion

Lipid Oxidation

Raw meat is highly susceptible to oxidative changes during production, handling, processing, and storage in refrigerated conditions (20). Several researchers have denoted that lipid oxidation in meats may be inhibited or decreased by natural antioxidant compounds (21-24). In the present study (Figure 1), on sampling days zero, two, four, six, eight, and ten, all the edible films (especially those enriched with 1% CE) showed inhibitory effects against lipid oxidation compared to the uncoated samples. This trend was more significant after 10 days of storage in the presence of a greater advancing of the process.

The initial PV was 0.57 meq/1,000 g of fat and reached 1.89 meq/1,000 g of fat in the unpacked samples after 10 days of storage. The changes in PV during storage at refrigerated temperature are consistent with the findings of other researchers in this regard (25-27). In addition, the PVs of pure CH and CH-GE continuously increased and reached 1.45 and 1.67 meq/1,000 g of fat at the end of the study, respectively. On the other hand, the addition of

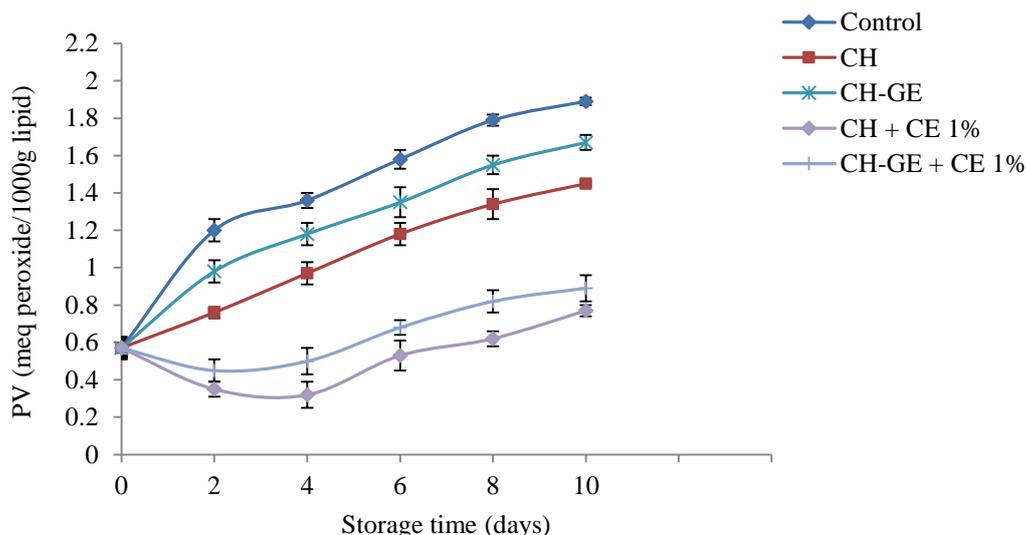


Figure 1. Effect of chitosan (CH), chitosan-gelatin (CH-GE) films and same films containing cinnamon extract (CE 1%) on peroxide value (PV) of chicken meat hamburger

1% CE to the films decreased the PV compared to the coated samples without the extract.

Accordingly, the values reached 0.77 and 0.89 meq/1,000 g of fat for CH and 1% CE and CH-GE and 1% CE, respectively. Our findings indicated the antioxidant chemical effectiveness of the natural constituents of CE. In this regard, Yuan et al. (23) reported that the antioxidant properties of CH could be due to the ability of the residual free amino groups of polysaccharide reacting with free radicals, thereby forming stable macromolecular radicals and ammonium groups. Moreover, Ojagh et al. (2010) denoted that cinnamon contains cinnamaldehyde, beta-caryophyllene, linalool, and other terpenes, which have recently been reported to be the cause of the antioxidant properties of cinnamon (28). Therefore, these compounds may largely contribute to the total antioxidant capacity of CE and its infusions.

In another research, Lekjing (2016) investigated PV in a lipid fraction of cooked pork sausages during 25 days of storage at refrigerated temperature, stating that the optimal antioxidant effects were observed with CH combined with clove oil. This could be due to the fact that CH acts as a chelator of the transition metal ions that initiate lipid peroxidation and chain reactions (22). Furthermore, Shavisi et al. (2017) claimed that the PVs of untreated beef meats were approximately 1-2 meq/kg of fat higher

compared to the samples packed with polylactic acid containing 2% *Ziziphora clinopodioides* essential oil and 2% propolis extract after 11 days of refrigerated storage (20).

Microbial Counts

In addition to lipid oxidation, meat spoilage may occur due to the microbial activities of various gram-positive and gram-negative bacteria since the nutrient composition, pH (5.5-6.5), and high moisture content of meat enable the growth and survival of microorganisms (29). The effects of various films with or without 1% CE on the population of total aerobic microorganisms, coliforms, and *S. aureus* at the temperature of 4°C are depicted in Figures 2-4.

In the present study, the initial population of the total aerobic microorganisms was 4.39 log CFU/g (day zero) (Figure 2). After 10 days of refrigerated storage, the CH-based films with the additional 1% CE maintained a slight microbial load compared to the first day ($P>0.05$). The final total aerobic counts of the controls and samples packed with pure CH, pure CH-GE, CH-GE and 1% CE, and CH and 1% CE were 8.99, 7.02, 7.91, 5.62, and 5.36 log CFU/g, respectively. As depicted in the Figure, the blended films displayed a reduction in terms of growth inhibition in the control samples, while significantly higher values than the straight CH film were also observed ($P<0.05$). Nevertheless, the initial microbial load of the coliform

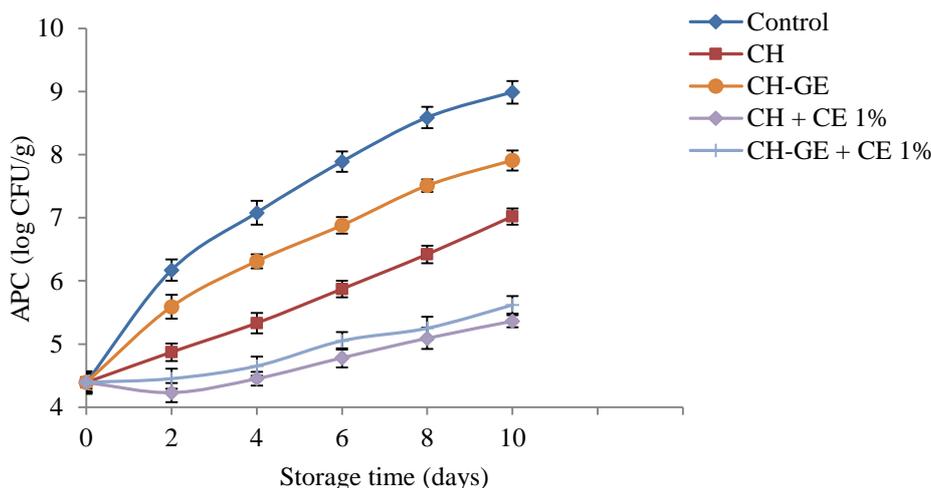


Figure 2. Effect of chitosan (CH), chitosan-gelatin (CH-GE) films and same films containing cinnamon extract (CE 1%) on total aerobic plate count (APC) of chicken meat hamburger

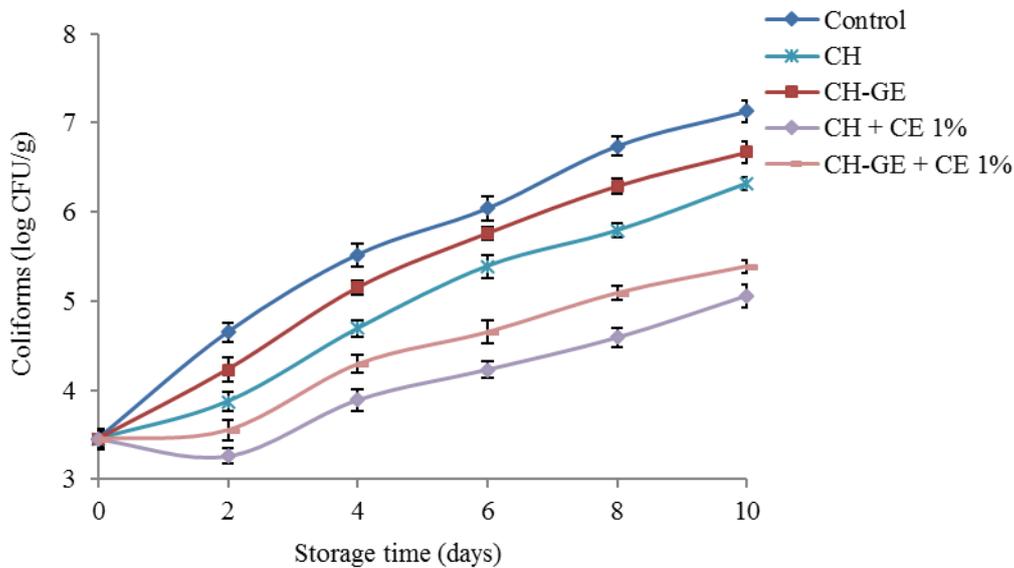


Figure 3. Effect of chitosan (CH), chitosan-gelatin (CH-GE) films and same films containing cinnamon extract (CE 1%) on coliform count of chicken meat hamburger

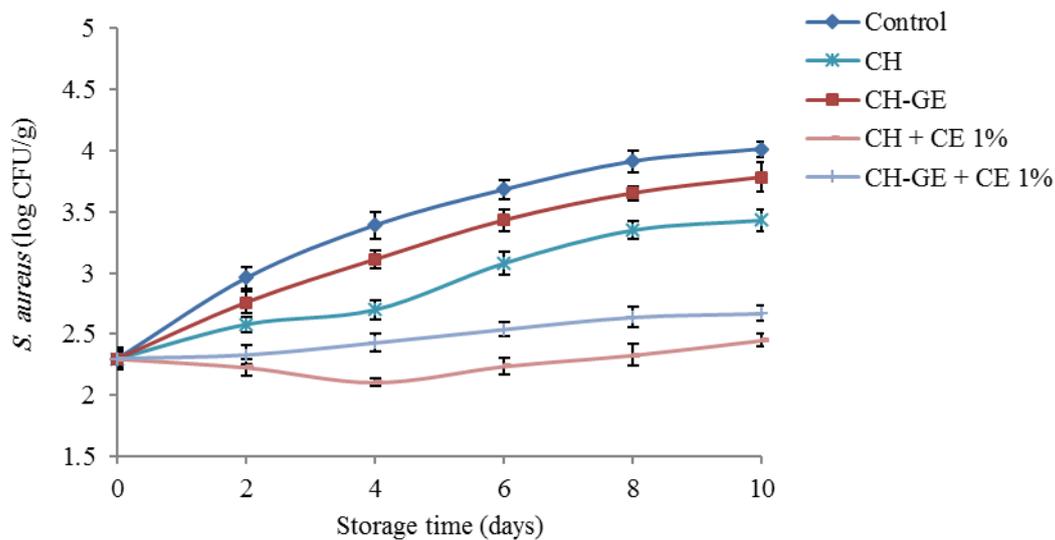


Figure 4. Effect of chitosan (CH), chitosan-gelatin (CH-GE) films and same films containing cinnamon extract (CE 1%) on *S. aureus* count of chicken meat hamburger

microorganisms was estimated at 3.45 log CFU/g (Figure 3).

After 10 days of storage, all the films could effectively decrease the population of coliforms in the chicken hamburger samples compared to the controls ($P < 0.05$). In other words, the final microbial load of the coliform microorganisms of the controls and samples packed with pure CH, pure CH-GE, CH-GE and 1% CE, and CH and 1% CE was estimated at 7.13, 6.32, 6.67, 5.39,

and 5.06 log CFU/g, respectively. As described previously, the pure CH-based films (with or without the addition of 1% CE) exhibited lower microbial load values compared to the blended films after 10 days of storage. Our findings indicated that the antimicrobial properties of the films were directly associated with CH concentration, the antibacterial properties of which have been confirmed in the previous studies regarding fresh meat and meat products

(30-33).

Finally, the initial microbial load of the *S. aureus* microorganism was 2.3 log CFU/g (Figure 4). After 10 days of refrigerated storage, the differences in the antimicrobial effects of all the films were described. According to the findings, the CH-based films and blended films with 1% CE maintained the initial microbiological load of *S. aureus* by 2.67 and 2.45 log CFU/g at the end of the study, respectively. Moreover, the present study showed that reducing the ratio of CH by 50% altered the effectiveness of the antimicrobial activity of the blended films, which was improved by the addition of 1% CE.

In general, our findings reflected that CH (alone or in the presence of additional CE) could retain remarkable antimicrobial activity in chicken hamburgers, and the presence of GE only contributed to improving the preservation quality due to its antioxidant activity, along with the incorporation of 1% CE. In their research, Khanjari et al. (2013) prepared edible CH-based films using oregano essential oil in order to assess their antimicrobial effects on raw chicken meat fillets (34). Therefore, they concluded that the addition of the essential oil to the CH-based films enhanced their antimicrobial properties, suggesting that the essential oil exerted less significant antimicrobial effects against the natural microbial population of meat compared to CH. Moreover, they observed that the CH-based films were the most efficient agents in the growth inhibition of *Listeria monocytogenes* throughout the storage period. Similarly, Nowzari et al. (2013) reported that CH-GE coating exhibited a remarkable inhibitory capacity in the microbial load of chilled rainbow trout fillets ($P < 0.05$) (35).

In another study, Dehnad et al. (2014) stated that the application of CH-based films decreased the population of spoilage bacteria by 1 log CFU/g in ground meat compared to nylon-packaged samples (36). Other studies in this regard have denoted that the antibacterial effects and numerous other benefits of cinnamon are associated with its major constituents (e.g., cinnamaldehyde, methoxycinnamaldehyde, and eugenol), which could extend heat shock protein (HSP) 60 and reduce the gradient across the cell membrane, thereby collapsing the proton motive force and leading to bacterial death (10, 11, 17,

37). Moreover, Mild et al. (2011) evaluated the protective effects of apple-based films enriched with cinnamaldehyde against antibiotic resistant (D28a and H2a) and susceptible *Campylobacter jejuni* strains (A24a) on chicken breast (38). According to their findings, the films containing 1.5% and 3% of cinnamaldehyde decreased the counts of all the strains within the ranges of 0.2-2.5 and 1.8-6 log CFU/g, respectively after three days of chilled storage.

Conclusion

According to the results, the combination of the CH-GE blend films with 1% CE delayed microbial population growth and decreased lipid oxidation, extending the shelf life of chicken hamburgers at refrigerated storage ($4 \pm 1^\circ\text{C}$). Moreover, the films of both the pure biopolymer films exhibited lower microbial population compared to the unpacked samples. The film-coated hamburger samples containing additional 1% CE displayed lower PV values at any storage time. Considering the obtained results, edible bioactive packaging films containing CH- and GE-based polymers incorporated with 1% CE could be used as an effective alternative for improving the preservation of chicken hamburgers.

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Conflict of interest

None declared.

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