



Packaging of Chicken Fillets with Active Chitosan-Poly (Vinyl Alcohol) Film Incorporated with *Zingiber Officinale* Extract: An Assessment of Shelf-Life Indices

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Research Paper</p> <hr/> <p><i>Article History:</i> Received: 04 Sep 2024 Accepted: 29 Oct 2024 Published: 16 Nov 2024</p> <hr/> <p><i>Keywords:</i> Shelf-life improving Antimicrobial packaging <i>Zingiber officinale</i> extract</p>	<p>Introduction: The aims of the present work were to add 0.25% and 0.5% <i>Zingiber officinale</i> extract into the chitosan-poly(vinyl alcohol) film by casting method and investigate their utilization as antimicrobial packaging polymer in retarding the chemical spoilage and microbial growth during prolonged shelf-life storage of chicken fillets.</p> <p>Methods: Fresh chickens were purchased from a local poultry processing plant (Kermanshah, Iran). One-hundred grams of chicken fillet samples were packaged with designated films and maintained at 4 ± 1 °C for 10 days. Total viable count, psychrotrophic bacterial count, <i>Enterobacteriaceae</i>, and peroxide value of chicken fillets were evaluated during refrigerated storage conditions.</p> <p>Results: The microbial counts and peroxide value of all prepared treatments significantly incremented over a 10-day storage time, while the growth rate of microorganisms and chemical changes in the control and fillets packaged with straight chitosan-poly(vinyl alcohol) film were higher compared to other treatments. Total viable count, psychrotrophic bacterial count, <i>Enterobacteriaceae</i>, and peroxide value of chicken fillets packaged with chitosan-poly(vinyl alcohol) film + 0.5% <i>Zingiber officinale</i> extract, as the best treatment, were 6.73 log CFU/g, 3.95 log CFU/g, 3.89 log CFU/g, and 0.82 meq peroxide/kg lipid, respectively, at the end of the study period.</p> <p>Conclusion: The results indicated that the designated films could be a promising method to extend the shelf-life of chicken fillets' quality during refrigerated storage.</p>
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Introduction

Chicken fillets are the most prevalent food products worldwide owing to their abundant nutrition, including vitamins, minerals, proteins, low lipid content, and relatively high concentration of polyunsaturated fatty acids (1, 2). However, it can be considered as a highly perishable food product because of its high moisture content and a rich source of nutrients, resulting in a very short shelf-life (3). Chicken spoilage mainly occurred owing to the physical, chemical, and microorganism proteolytic activities, which results in the loss of nutrition and food deterioration along with harm to consumer health (1). Packaging along with refrigerated storage conditions (approximately 0-4 °C) can be considered one of the most common approaches to protect food products from microbial and biochemical spoilage and

environmental hazards (4). In the last decades, the application of antimicrobial packaging materials containing plant essential oils/extracts has been presented for preserving the quality and extending the shelf-life of poultry meat products (5). The recent experiments confirmed the effectiveness of biodegradable/edible polymers to increase the shelf-life of chicken breast meat, such as chitosan (CS) film + lemongrass essential oil (6), low-density polyethylene film + clove essential oil (7), CS-montmorillonite film + rosemary essential oil (8), and CS film + ginger essential oil (9). Among polymers, poly(vinyl alcohol) (PVA) $[\text{CH}_2\text{CH}(\text{OH})]_n$ as a water-soluble polymer has biodegradable, biocompatible, and processability characteristics (10). PVA is one of the few synthetic biodegradable polymers with good film-forming properties (11). Given that

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PVA presents excellent mechanical, chemical resistance, and weight-bearing properties it can be widely utilized for food packaging applications (12). The non-toxicity, compatibility with biological systems, and intrinsic antimicrobial activity of CS have also been exploited to protect food from antimicrobial contamination, and therefore blending of CS with PVA is a convenient approach to improve the physical and mechanical properties of the prepared polymers for its potential applications in the field of food packaging (11).

At present, the meat industry utilizes chemical preservatives in several meat processes to retard the growth of bacterial pathogens and improve the shelf-life of chilled chicken fillets (13). Due to the concern over the safety of chemical preservatives, consumers increasingly demand the usage of natural additives as alternative preservatives in food packaging (14). *Zingiber officinale* root as a rich source of α -zingiberene, zingiberol, shogaol, and 6-gingerol, is related to the numerous health benefits, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and antidiabetic activities, which lead to its wide application in a variety of commercial natural products and antimicrobial packaging polymers (9, 15). *Z. officinale* essential oil/extract has been commonly applied in nutritional and pharmaceutical applications as a natural remedy and a flavor enhancer in food products (16, 17). Antimicrobial activity of *Z. officinale* against *Listeria monocytogenes* (18), *Staphylococcus aureus* (19), *Bacillus cereus*, and *Bacillus subtilis* (20) have been previously reported. To the best of our knowledge, the fabrication of active films treated with *Z. officinale* extract (ZOE) has not been evaluated. Therefore, the aims of the present work were to add ZOE 0.25% and 0.5% into the CS-PVA film by casting method and investigate their utilization as antimicrobial packaging polymer in retarding the chemical spoilage and microbial growth during prolonged shelf-life storage of chicken fillets.

Materials & Methods

Preparation of Zingiber Officinale Extract

Fresh ginger rhizomes (*Z. officinale*) were purchased from a local market, Kermanshah, Iran, washed, peeled, and chopped into the small pieces. An amount of 25 g of ginger rhizomes was milled into a fine powder (Moulinex, France), incorporated into the 100 ml distilled water, and

coated with aluminum foil. It was consecutively shaken at room temperature (25 ± 1 °C) for 24 h using a digital shaker (Behdad, Iran). Afterward, the mixture was filtered using a Whatman filter paper 41 and purified twice by centrifugation using a refrigerated centrifuge (Sigma, UK) at $12000 \times g$ for 10 min at refrigerated temperature (4 ± 1 °C). Finally, the solvent in the extract was removed using a rotary evaporator (Heidolph, Germany) at 40 ± 1 °C for 4 h and then the extract was vacuum-dried to achieve the anthocyanin-rich ZOE. The extract was kept at refrigerated conditions before further utilization. (21).

Preparation of Poly (Vinyl Alcohol)-Chitosan Film

Preparation of PVA-CS solution was conducted based on our preliminary experiment. Briefly, 3 g PVA powder (medium molecular weight = 72 KDa, purchased from Merck, Germany) was dissolved in 100 distilled water at 90 ± 1 °C under vigorous magnetic stirring (IKA, Germany) for 6 h. 1 g CS powder (medium molecular weight = 450 KDa, purchased from Merck, Germany) was dissolved in a 100 ml aqueous solution of acetic acid (1%, Merck, Germany) and stirred for 3 h at room temperature. Then, the above obtained solutions were mixed at a ratio of 1:2 to obtain a PVA-CS mixture under vigorous stirring for 2 h at room temperature. Afterward, different amounts of ZOE (including 0.25% and 0.5%) were added to the PVA-CS and mixed for another 2 h at room temperature. Glycerol (75 mg/100 ml) was added as a plasticizer in the above mixture to obtain designated films that were less brittle and easier to handle. Finally, 50 ml of the PVA-CS, PVA-CS + ZOE 0.25%, and PVA-CS + ZOE 0.5% solutions were poured into glass petri dishes with a diameter of 100 mm and allowed to dry at room temperature (22).

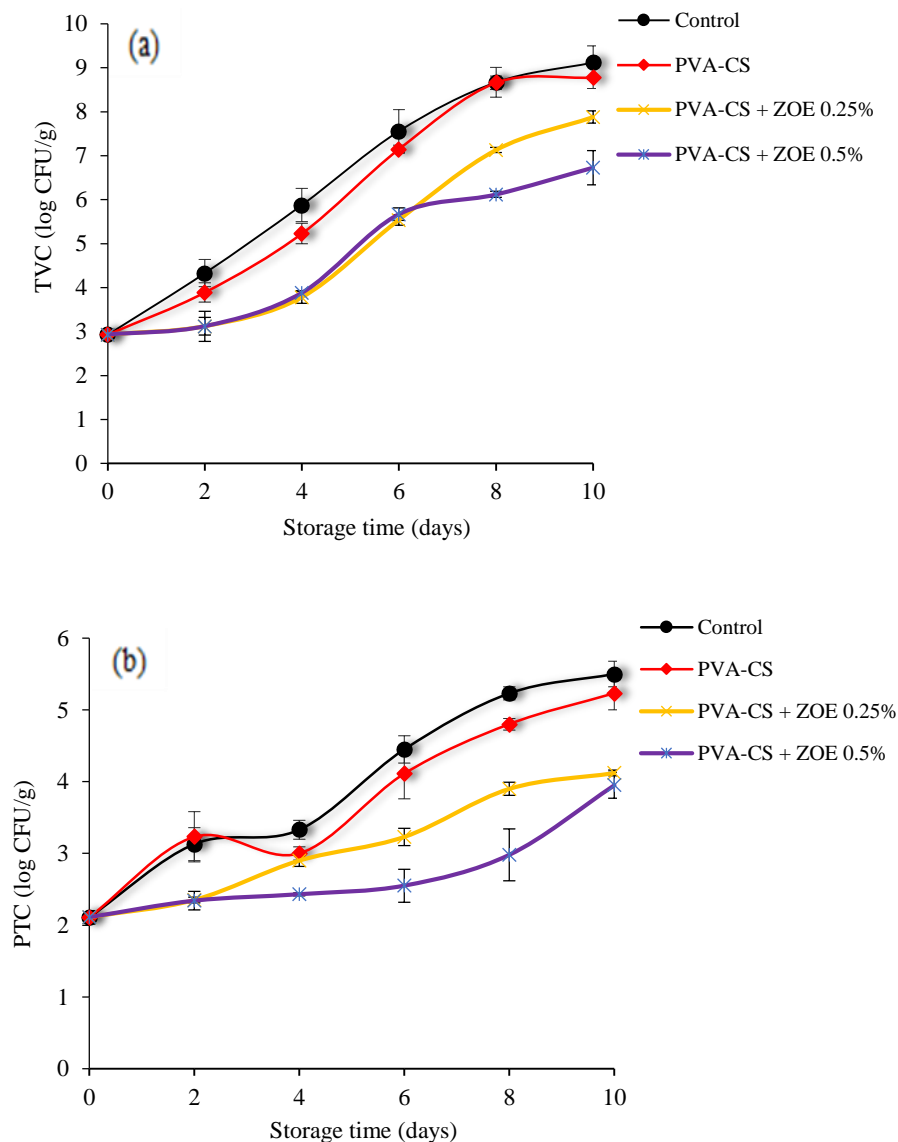
Packaging of Chicken Fillets

Fresh chickens were purchased from a local poultry processing plant (Kermanshah, Iran), placed in insulated polystyrene boxes on ice, and immediately transferred into the laboratory. Breast fillets were then aseptically cut into 25 g portions and stored at refrigerated conditions before further use. Afterward, 100 g of samples were packaged with designated films and maintained at 4 ± 1 °C for 10 days. Following the preservation period, the chicken fillets were taken, homogenized in the sterile 0.1% buffered peptone water, and cultured onto the plate count

agar (incubated at 37 ± 1 °C for 48 h for total viable count (TVC)), plate count agar (incubated at 7 ± 1 °C for 10 days for psychrotrophic bacterial count (PTC)), and violet red bile glucose agar (incubated at 37 ± 1 °C for 24 h for *Enterobacteriaceae*), respectively (4). The peroxide value (PV) of chicken fillets was also evaluated according to the previously published method by Majidinasab et al., (2020) (23).

Statistical Analysis

The study was performed three times. Data analysis was performed by employing a Tukey HSD test (SPSS 23, Chicago, IL, USA). The data were expressed as mean value \pm standard deviation. $P < 0.05$ was presented as the minimal level of statistical significance.



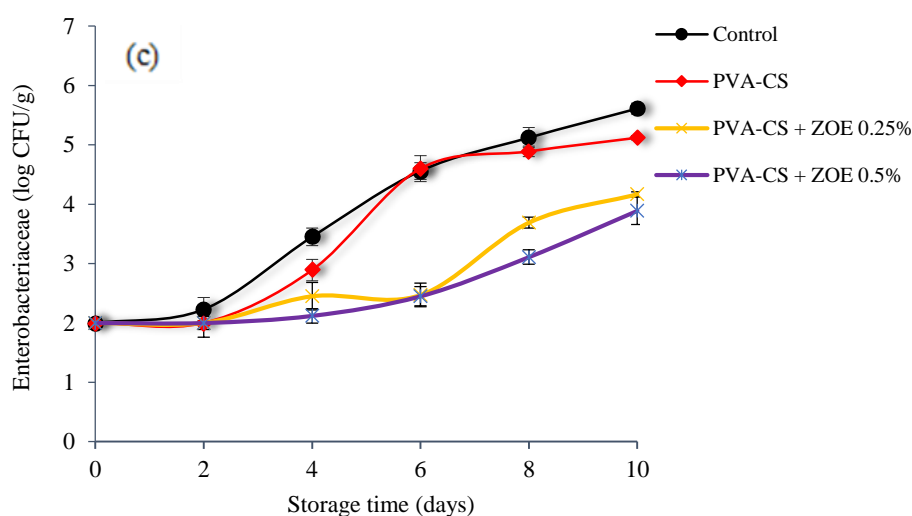


Figure 1. Microbial changes, including TVC (total viable count, a), PTC (psychrotrophic bacterial count, b), and *Enterobacteriaceae* (c), of chicken fillets during refrigerated storage conditions. Data are presented as mean \pm standard deviation.

Results & Discussion

The changes in the microbial population of chicken fillets, including TVC, PTC, and *Enterobacteriaceae*, during the 10-day storage period, are presented in figure 1a-c, respectively. The TVC, PTC, and *Enterobacteriaceae* of fresh chicken fillets were 2.93, 2.11, and 2.0 log CFU/g at day 0, respectively. The microbial counts of all prepared treatments significantly incremented over a 10-day storage time, while the growth rate of microorganisms in the control and fillets packaged with straight PVA-CS film were significantly higher comparing with other treatments ($P < 0.05$). According to the acceptable limits of 7, 7, and 5 log CFU/g for TVC, PTC, and *Enterobacteriaceae*, the control and chicken fillets packaged with PVA-CS film were spoiled after 6 days of chilled storage conditions. A similar finding has been indicated by Kanatt, (2020) (24), who found that the increase in TVC in chicken fillets in PVA-gelatin film was comparatively slower than in the control group. The lower microbial population of PVA-CS film in comparison with the control group might be attributed to the interactions between positively charged amino groups of CS and negatively charged anionic cell surfaces, resulting in incrementing outer cell membranes permeability, and finally cell death (25). Jonaidi Jafari et al., (2018) reported that CS coating-propolis extract was effective in retarding the growth of microbial population, including TVC, PTC, and lactic acid bacterial count, of chicken fillets during the 12-day refrigerated storage

period (26). In another study (1), the combination of *Syzygium aromaticum*, *Cinnmorum cassia*, and *Origanum vulgare* extracts effectively controlled the growth of spoilage microorganisms in chilled chicken fillets. Jridi et al., (2018) also found that the incorporation of henna extract as a natural antimicrobial compound into the gelatin coating provided a superior protection against microbial population growth in beef meat (27). Takma et al., (2019) reported that CS and alginate coatings incorporating black cumin oil were effective in retarding the growth of aerobic mesophilic and psychrotrophic bacteria in the chicken breast meat (3). As given in figure 1a-c, TVC, PTC, and *Enterobacteriaceae* of chicken fillets packaged with PVA-CS + ZOE 0.5% film, as the best treatment, were 6.73, 3.95, and 3.89 log CFU/g, respectively. It has been reported that *Zingiber officinale* contains monoterpenoids, sesquiterpenoids, phenolic compounds, and its derivatives, aldehydes, ketones, alcohols, and esters, which provide a broad antimicrobial spectrum against different microorganisms in food models and *in vitro* conditions (15, 28). PV as the primary lipid oxidation index is utilized to determine the development of peroxides and hydroperoxides in the initial stages of lipid oxidation (4). Decomposition of chemically active hydroperoxides resulted in the development of secondary oxidative compounds, and undesirable odor and flavor of chicken meat. As presented in figure 2, the initial PV of fresh chicken meat samples was determined to be 0.52

meq peroxide/kg lipid. The PV of unpackaged samples and fillets packaged with PVA-CS films significantly increased with storage time and reached 1.22 and 1.12 meq peroxide/kg lipid, respectively, after 10 days of refrigerated storage conditions. Lipid oxidation decay of chicken fillets packaged with straight PVA-CS film was delayed compared to the control group. It can be related to the low oxygen barrier characteristic of fabricated film compared to the control group (23). The lowest PV was significantly found in chicken fillets packaged with PVA-CS + ZOE

0.25% and PVA-CS + ZOE 0.5% films at the end of the experiment ($P < 0.05$), recorded by 0.95 and 0.82 meq peroxide/kg lipid, respectively (figure 2), probably due to the antioxidant activity of ZOE, which is rich in polyphenolic compounds, as previously reported (29-31). The lower PV of treated samples could be related to the neutralization of any free radicals by electrons of anthocyanin extracts, decomposition of hydroperoxides, and interaction with transition metals (32).

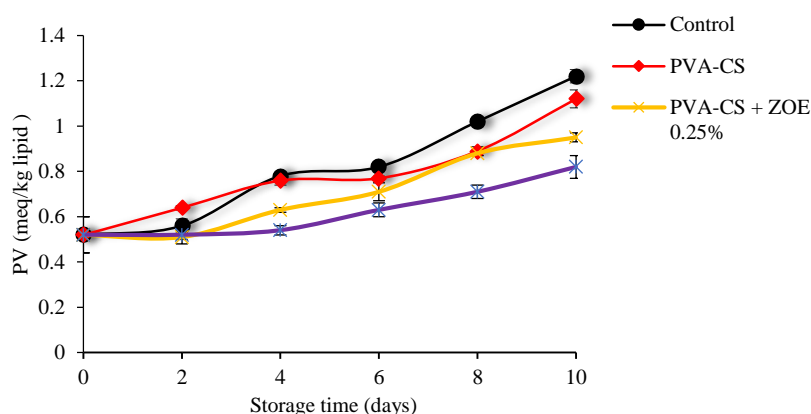


Figure 2. Peroxide value of chicken fillets during refrigerated storage conditions. Data are presented as mean \pm standard deviation.

Conclusion

Our findings showed that the antioxidant and antimicrobial activities of designated films resulted in minimizing the oxidative effects, retarding the microbial growth, and incrementing the shelf-life of fresh chicken fillets for ten days. However, there were some limitation aspects in this experiment, particularly evaluation of sensory properties of the treated chicken fillets with PVA-CS + ZOE 0.25% and PVA-CS + ZOE 0.5% films along with *in vitro* examination of antioxidant characteristic of developed films.

Declarations

Acknowledgements

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Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Negin Karami: Investigation; Nassim Shavisi: Conceptualization; Methodology; Formal analysis; Writing-Original Draft.

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