

Development of antimicrobial bioactive packaging based on plant essential oils to prevent contamination of cherries with human pathogens

Amirhossein Rezazadeh Shirazi*

PhD student in General Veterinary Medicine,

Kazerun Azad University, Fars, Iran

amirrezazadehshirazi1347@gmail.com

Ahmadreza Keshtgar

Bachelor of Science in Microbiology, Islamic Azad

University, Kazerun Branch, Fars, Iran

rezauou3@gmail.com

Hanieh Salehi

Bachelor of Science in Microbiology, Islamic Azad

University, Kazerun Branch, Fars, Iran

hanisalehi751@gmail.com

Changiz Kameli

PhD in General Veterinary Medicine, Kazerun Azad

University, Fars, Iran.siamkk53@gmail.com

Abstract

Background and Objective: Microbial contamination of fresh fruits, especially cherries, is a major concern in the field of food safety and public health. The aim of this study was to develop bioactive packaging containing plant essential oils with antimicrobial properties and evaluate their efficacy in inhibiting the growth of human pathogens on the surface of cherries. **Materials and Methods:** In this experimental study, 10 cherry samples from 5 different farms (2 samples from each farm) were collected and packaged in 5 types of bioactive films impregnated with natural essential oils (thyme, cinnamon, rosemary, garlic, and eucalyptus). The samples were stored at refrigerator temperature for 7 days. Microbiological tests including total bacterial count (TVC), Enterobacteriaceae count, and detection of *E. coli* and *Salmonella* spp. were performed before and after packaging. **Findings:** The results showed that packaging containing thyme and cinnamon essential oils had the most significant reduction ($p < 0.01$) in microbial load, especially in the elimination of Enterobacteriaceae and the inhibition of *E. coli* growth. Also, a significant decrease in the number of colonies was observed on day 7 compared to day 0.

Conclusion: Bioactive packaging based on plant essential oil can be used as an effective, safe and sustainable solution to reduce cherry contamination and improve food safety. This method has the potential to be used in the fruit packaging industry with the aim of preventing the transmission of human pathogens.

Keywords: Bioactive packaging; plant essential oil; microbial contamination; cherry; human pathogens

Introduction

In recent decades, concerns about the safety and quality of food, especially fresh fruits and vegetables, have increased significantly. One of the main challenges in the supply chain of these products is the control of microbial contamination, especially human pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes*, which can be transmitted to fruits through contact with contaminated surfaces, water, soil, or packaging processes (Alonso et al., 2024; Casalini et al., 2023).

Among horticultural products, cherries are susceptible to microbial contamination and spoilage due to their soft texture and moist surface. Raw consumption of this fruit increases the possibility of transmitting pathogens to humans. Also, the short shelf life of this product highlights the need for innovative solutions to maintain its quality and safety (Kapetanakou et al., 2024).

One of the new strategies in this area is the use of bioactive packaging technology, which not only plays a protective role but also has the ability to release antimicrobial compounds during product storage (Mohan & Panneerselvam, 2022). In this regard, plant essential oils have been proposed as an effective alternative to chemical preservatives due to their antimicrobial properties, biodegradability, and safety of consumption (Guarda et al., 2024; Tyagi et al., 2012). Essential oils such as thyme, cinnamon, rosemary, garlic, and eucalyptus have active compounds such as thymol, carvacrol, allicin, and 1,8-cineole that have shown prominent inhibitory effects against foodborne pathogens by destroying bacterial cell walls or disrupting their metabolism (Casalini et al., 2023; Tyagi et al., 2012). These compounds

can be incorporated into packaging films based on natural polymers such as starch, chitosan or PLA to provide a controlled delivery system (Mohan & Panneerselvam, 2022).

Several studies have investigated the effectiveness of bioactive coatings containing plant essential oils in controlling the growth of microorganisms on fruits such as strawberries, raspberries, tomatoes and apples (Kapetanakou et al., 2024; Noshirvani & Le Coz, 2024). For example, chitosan films containing thyme essential oil have been able to reduce the growth of *E. coli* and *S. aureus* bacteria by more than 90% (Casalini et al., 2023). Also, the use of bioactive packaging with the ability to release essential oil vapor has made it possible to increase the shelf life of fruits (Tyagi et al., 2012). Despite promising results, specific research on cherries as hosts for human pathogens has been limited. Also, investigating the efficacy of bioactive films on a semi-industrial scale, using field data from several farms, and measuring performance under real storage conditions (cold chain) are among the research gaps in this area (Guarda et al., 2024; Wyrwa & Barska, 2017).

Accordingly, the present study aimed to design, produce, and evaluate bioactive films impregnated with plant essential oils for use in cherry packaging. This study used 10 cherry samples harvested from 5 farms and different packaging. The main objective was to investigate the ability of this packaging system to reduce microbial load and human pathogens during storage.

The specific objectives of the study are as follows:

1. Design and production of 5 types of bioactive films containing selected plant essential oils.
2. Evaluation of the physical, chemical and release properties of the films.
3. Measurement of the antimicrobial effectiveness of the films on cherries contaminated with human pathogens.
4. Comparison of the results under cold storage conditions with common market packaging.

Results

A total of 10 cherry samples from 5 different distribution and packaging centers (Centers A to E) were evaluated. Initial microbial analysis

Given the increasing need for sustainable and safe systems for storing fresh fruits, it is expected that the findings of this research can play an effective step towards improving the safety and quality of the cherry supply chain at the national and global levels.

Methods

In this study, 10 cherry samples were collected from 5 different farms in the Kazerun region (Fars province) and from two stages of the supply chain (farm and final packaging) in a systematic random manner. Sampling was carried out in two times (morning and evening) during the official harvest period of the season. Each sample contained about 500 grams of ripe and healthy cherries, which were placed in sterile containers and transported to the laboratory under cold conditions (4 °C).

To prepare the bioactive packaging, five types of biodegradable films based on polylactic acid (PLA) or chitosan were used, which were coated with natural essential oils (cinnamon, thyme, garlic, rosemary and eucalyptus) by thermal spraying and vacuum fixation. The amount of essential oil used varied between 1 and 2% of the film's dry weight, and the physicochemical properties of each film, including thickness, elasticity, water vapor permeability and essential oil release rate, were measured.

To investigate microbial contamination, cherries were first washed, homogenized, and cultured in nutrient agar and MacConkey agar using the ISO 6887 standard method. Total aerobic bacteria, coliforms, and specific pathogens including *E. coli*, *Salmonella* spp., and *Listeria monocytogenes* were counted. Then, each cherry sample was packaged and stored for 7 days at 4°C, and microbial evaluation was performed on days 0, 3, and 7.

Packaging films were applied in two ways: direct and indirect contact (essential oil vapor). The control group was uncoated or with neutral film (without essential oil). Statistical analysis of data was performed with SPSS software using ANOVA and Tukey test (significance level $p < 0.05$).

showed that the average total microbial load on day 0 (before packaging treatment) ranged from 4.2 to 5.7 Log CFU/g. The highest contamination was recorded in samples collected

from Packaging Center D, while Center A showed the lowest initial load. After applying bioactive packaging containing plant essential oils and storing for 7 days at 4°C, the results showed that all packaging formulas caused a

significant reduction ($p < 0.05$) in the total microbial load and the number of pathogenic bacteria. The table below shows the average reduction in microbial load on day 7 by essential oil and center:

Reduction in the average total microbial load (Log CFU/g) on day 7:

Center	Thyme	Cinnamon	Garlic	Rosemary	Eucalyptus
A	2.3	2.6	2.9	2.1	1.8
B	2.5	2.8	3.0	2.3	2.0
C	2.1	2.4	2.7	2.0	1.6
D	2.8	3.2	3.5	2.6	2.4
E	2.4	2.7	3.1	2.2	1.9

The greatest reduction in coliform and *E. coli* bacterial load was observed with garlic essential oil treatment (mean reduction of 3.04 Log CFU/g). Cinnamon essential oil also produced a significant reduction in *Listeria monocytogenes* population (mean reduction of 2.9 Log CFU/g), while thyme essential oil was very effective in controlling the growth of *Salmonella* spp. (mean reduction of 2.6 Log CFU/g). In the control group (without coating or with neutral film), microbial growth continued in most samples and in some cases reached more than 6.2 Log CFU/g, indicating the poor effectiveness of conventional packaging in preventing the growth of pathogens.

Comparison between centers showed that the effectiveness of bioactive packaging was also significant in centers with higher initial contamination (especially center D) and showed a similar reduction rate to other centers. This indicates the stability of the antimicrobial performance of the packaging in different initial product conditions. In terms of appearance indicators (color, texture, and cherry odor), thyme and cinnamon essential oil treatments had the highest preservation of sensory quality, while

garlic essential oil, despite its high antimicrobial effectiveness, caused noticeable olfactory changes in some samples.

Discussion

The findings of this study showed that bioactive packaging based on plant essential oils can effectively reduce microbial contamination of cherries throughout the distribution and storage chain. Essential oils such as garlic, cinnamon and thyme played a prominent role in controlling the growth of pathogenic bacteria, through mechanisms such as disruption of cell wall structure, reduction of membrane permeability and inhibition of bacterial metabolic pathways (Burt, 2004; Hyldgaard et al., 2012).

The results are consistent with previous studies that have confirmed the effectiveness of plant essential oils in controlling foodborne microorganisms. For example, Kapetanakou et al. (2024) in a study on cherry tomatoes showed that bioactive packaging containing carvacrol was

able to significantly inhibit the growth of *Salmonella enterica*. Similarly, Guarda et al. (2011) and Mohan & Panneerselvam (2022) have also demonstrated the effectiveness of rosemary and cinnamon essential oils in maintaining fruit quality during storage.

Notably, in the present study, the high effectiveness was observed even in samples with high initial contamination (center D), indicating the stability of the antimicrobial performance of these packages. It was also observed that packages with garlic essential oil had the highest inhibitory effect on *E. coli* and coliforms, which is consistent with the study of Bagamboula et al. (2004) and Nazzaro et al. (2013).

In addition to antimicrobial effects, organoleptic considerations should also be considered. Despite its high effectiveness, garlic essential oil caused a change in the cherry odor, which may reduce consumer acceptance. In this regard, combining essential oils or using nanoencapsulation for controlled release could be a suitable solution (Donsi & Ferrari, 2016).

Limitations of the present study include the limited number of samples and the lack of accurate molecular assessments to identify microbial resistance genes. It is also necessary to investigate consumer response to sensory changes in future studies.

Conclusion

This study demonstrated that bioactive packaging containing plant essential oils can be an effective, safe, and environmentally friendly approach to reduce the microbial load of cherries throughout the supply chain. Among the essential oils studied, garlic essential oil had the highest antimicrobial effect, while cinnamon and thyme essential oils also performed significantly while maintaining sensory properties.

The implementation of this type of packaging at an industrial level could play a key role in reducing post-harvest losses, increasing food safety, and reducing the need for chemical

preservatives. It is suggested that future research investigate the efficacy of these packaging on a larger scale, in other horticultural products, and with nanoencapsulation and controlled release methods.

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Conflict of Interest

The authors of this article declare that there is no scientific, financial, or personal conflict of interest in relation to the subject of this research.

Data Availability

All data obtained from this research can be provided by the authors of the article in a complete and documented form upon request and will be made available to interested researchers.

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