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Effect of Gelatin Coating Containing *Lactobacillus Rhamnosus* and *Bifidobacterium Bifidum* on Chicken Fillet Inoculated with *Listeria Monocytogenes*

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ABSTRACT

Background: The purpose of this study is to investigate the effects of gelatin coating containing *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* on *Listeria monocytogenesis* inoculated in chicken fillets during cold storage (4 ± 1°C).
Methods: After inoculation with *L. monocytogenes* bacteria, the samples were divided into three groups: uncoated fillets (control), fillets packaged with gelatin coating, and fillets coated with *L. rhamnosus* and *B. bifidum*. Then, the samples were transferred to the refrigerator to check the viability of probiotics and count *L. monocytogenes* on days 0, 3, 6, 9, 12, and 15.
Results: The results of the viability showed a significant difference between the control samples and those coated with *L. rhamnosus* and *B. bifidum* ($P < 0.05$). Regarding the control of *L. monocytogenes* growth, the results indicated a significant inhibitory effect on this bacterium compared to the control group ($P < 0.05$). Moreover, the sensory evaluation characteristics of chicken fillets showed that the edible coating did not have an adverse effect on sensory properties, and the product was preserved.
Conclusion: The gelatin edible coating can act as a suitable matrix for a combination of probiotic bacteria in foods to control pathogens.

1. Introduction

All kinds of meat, particularly chicken, are a popular food choice and a primary source of animal protein for many people worldwide. Its consumption has risen over the past few decades (Dave & Ghaly, 2011). Some of the reasons for the popularity of this product are its relatively low production cost, low-fat content, and high nutritional value (Gallocchio et al., 2016). Nowadays, the disease caused by the consumption of food contaminated with pathogenic bacteria has become a public concern. It seems that to reduce health-threatening cases, the use of natural products is an appropriate way to control pathogenic bacteria such as Salmonella, *Escherichia coli*, *Campylobacter jejuni*, *L. monocytogenes* and also to extend the shelf life of processed foods (Azizian et al., 2019; Dharod et al., 2007). Although *L. monocytogenes* is a common food pathogen in cheese and meat products, *L. monocytogenes* can also be encountered in chicken. Frozen chicken croquettes and chicken sausages are separated. The presence of *L. monocytogenes* in poultry products, especially raw meat, is considered a serious threat due to the possibility of cross-contamination with other foods both in retail environments and in home refrigerators (Kazemeini et al., 2021). Edible films and coatings based on polysaccharides, proteins, lipids, or a combination of them, in addition to solving environmental and economic problems, are used as carriers of additives such as antimicrobials and antioxidants. Edible coatings can improve the safety, durability, quality, and sensory characteristics of food (Kazemeini et al., 2021; Kazemeini et al., 2019). Gelatin is a food protein that is mainly obtained from the processing of collagen, which is the main structure of the skin, tendons,

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bones, and other parts of vertebrates. Also, with the properties of adhesion, crystallization, shaping, emulsification, and water absorption, it is used in a wide range of foods. Therefore, the use of gelatin coating and wrapping due to its naturalness and the above-mentioned features in the preservation of various food items has been the focus of researchers, and various studies have shown the positive effects of coverings and wrapping (Gómez-Estaca et al., 2009). Probiotics are living microorganisms that have beneficial health effects when consumed in sufficient amounts. Lactic acid bacteria and bifidobacteria are the most common probiotics used in dairy products (Banuree et al., 2022). The positive effects of probiotic bacteria have increased their use in a group of foods known as functional foods. Probiotic bacteria improve the microflora balance of the digestive system, strengthen the mucosal defense system against pathogens, increase immune responses, reduce blood cholesterol, and have anti-cancer and antimicrobial activity (Banuree et al., 2022; Soukoulis et al., 2016). According to what was said, this study aims to investigate the effect of gelatin coating containing *L. rhamnosus* and *B. bifidum* probiotics on chicken fillet inoculated with *L. monocytogenes* during a 15-day period kept at a cold temperature (4 ± 1°C) and also the chemical and sensory characteristics of chicken fillet have been investigated.

2. Materials and Methods

L. monocytogenes (ATCC19118), *L. rhamnosus* (PTCC 1637), and *B. bifidum* (PTCC 1644) lyophilized bacteria were obtained in boxes containing ice from the Iran Scientific and Industrial Research Organization. All culture media were purchased from Merck (Merck, Germany).

2.1 Preparation of bacteria

To prepare the bacteria under sterile conditions, 0.3 to 0.4 mL of sterile brain heart Infusion (BHI) broth culture medium was added to the dry material inside the vial containing *L. monocytogenes* and homogenized to form a uniform suspension. The prepared suspension was then transferred into a larger test tube containing 20 mL of BHI broth and cultured linearly on a nutrient agar culture medium (Merck, Germany). In the next step, the plate and tube containing *L. monocytogenes* were placed in an incubator at 37°C for 24 h. To adjust the number of bacteria per mL, the spectrophotometer was set to 600 wavelengths (The amount of optical absorption equal to 0.08 to 0.133 at the wavelength of 600 nm) and an approximate number of 1.5×10^8 bacteria per mL was obtained (Kazemeini et al., 2021; Banuree et al., 2022).

2.2 Preparation of probiotic bacteria

Probiotic bacteria used in this study were sub-cultured in MRS broth (Merck, Germany) following two preparation cycles. Then, the second culture was mixed with sterile glycerol at a ratio of 4:1 and distributed in 500 µL volumes in sterile Eppendorf microtubes, and stored at -20°C. For inoculation, the bacterial cultures were collected by centrifugation (for 5 minutes at 5°C) and washed twice with Ringer's solution. The concentration of each probiotic culture was adjusted to 1×10^8 log CFU/mL until after adding to the CFU/g the concentration of log CFU/mL is obtained (Banuree et al., 2022; De Lacey et al., 2012).

2.3 Preparation of gelatin coating

Cow bone gelatin powder (Bloom Gelatin, Netherlands) was purchased for this study. A 1% gelatin solution was prepared by dissolving 1g of gelatin powder in 100 mL of distilled water at room temperature, stirring for 15 min until completely dissolved. Then, 0.15 g of glycerol and 0.015 g of sorbitol per gram of gelatin were added as plasticizers. The solution was stirred gently at 40°C for 15 min. Probiotic suspensions (1×10^8 log CFU/mL) were then added to the coating (Gómez-Estaca et al., 2009).

2.4 Preparation of chicken fillet and studied treatments

Fresh chicken fillets were procured from the market in sufficient quantities on the slaughter date and transported to the laboratory in boxes containing ice. The fillets weighing 50 g were prepared for treatment by washing, draining in sterilized plastic colanders, and exposing them to UV rays to remove excess water. They were then stored beside ice to prevent contamination with *L. monocytogenes*. A sample of 25 g of chicken fillet was added to 225 mL of LEB broth and kept for 48 h at a temperature of 30°C. Following this, 0.1 mL of the culture was cultured on the surface of Palckam agar culture medium (Merck, Germany) and incubated (Kazemeini et al., 2021) at 30°C for 48 h. The inoculated samples were divided into four groups (Table 1) for treatment. The samples were immersed in the 1% gelatin solution for 20 min, drained, and stored in sterile zip bags at 4±1°C for 15 days. Finally, analysis was performed on days 0, 3, 6, 9, 12, and 15 (Gallocchio et al., 2016; Kazemeini et al., 2021).

2.5 Probiotic bacteria count

To prepare the dilution, 10 g of homogenized chicken fillet was weighed in sterile zippered bags containing 90 mL of sterile peptone water (0.1 g in 100 mL of distilled water) and homogenized for 2 min by Stomaker (Bioxia, India). To prepare a dilution, one mL of the prepared dilution was added to a tube containing 9 mL of 0.1% sterile peptone water and mixed by a vortex. In the same way, successive dilutions were prepared and then 100 µL of each dilution was taken and cultured in the plates containing MRS agar and MRS agar containing 0.05% cysteine (Merck, Germany) in triplicate, then incubated for 48-72 h at 37°C under anaerobic conditions. The counting results were reported in terms of CFU/g after calculation (Banuree et al., 2022; Soukoulis et al., 2016).

2.6 Listeria monocytogenes bacteria count

First, 10 g of each sample was added to the streel bag containing 90 mL of sterile peptone water and mixed, and it was placed in the Stomaker (Bioxia, India) at a speed of 7 for

2 min to obtain a homogeneous suspension (dilution 1-10). Then, 1 mL of the upper surface of the suspension was removed with the help of a sampler and poured into a test tube containing 9 mL of sterile peptone water until a dilution of 2-10 was obtained (Kazemeini et al., 2021). After successive dilution of the desired dilutions, 100 µl were cultured on PALCAM listeria selective agar (Merck, Germany) and incubated at 37°C for 24 to 48 h for microbial counting (Kazemeini et al., 2021; Rezaeian et al., 2021).

2.7 Sensory evaluation

Sensory evaluations of the chicken fillets were conducted by a trained panel of nine people throughout the storage period. The parameters evaluated included aroma, texture, appearance, taste, and overall acceptance, using a scoring scale from 0 to 5, where 5 indicated the highest score and 0 represented an unacceptable product (Kazemeini et al., 2021).

2.8 Statistical analysis

Mean values, standard deviations, minimum, and maximum bacterial counts for each group and each day of the study were reported. The data was recorded in Excel software. SPSS software (IBM, USA) version 23 and ONE WAY ANOVA method were used for the statistical analysis of data with a p-value less than 0.05 considered statistically significant. An analysis of variance test was used to check the significant differences between the treatment and control groups. Duncan's statistical test was used to compare the difference between means.

Table 1. The different treatments on the chicken in this study

Row	Treatments	Description
1	Control	Gelatin chicken fillet pieces inoculated with *L. monocytogenes* (uncoated)
2	Gelatin	Chicken fillet pieces inoculated with *L. monocytogenes* + gelatin coating
3	Gelatin + *L. rhamnosus*	Chicken fillet pieces inoculated with *L. monocytogenes* + gelatin coating + *L. rhamnosus*
4	Gelatin + *B. bifidum*	Chicken fillet pieces inoculated with *L. monocytogenes* + gelatin coating + *B. bifidum*

3. Results and Discussion

As shown in Figure 1, during the storage period of the samples at the refrigerator temperature, the number of probiotic bacteria increased significantly, with higher levels in *L. rhamnosus* bacteria than in *B. bifidum*. Specifically, the counts of *L. rhamnosus* and *B. bifidum* in the gelatin-coated treatments were 7.98 and 8.01 log CFU/g respectively, on day zero and this amount increased to 11.57 and 9.94 log CFU/g by day fifteen ($P < 0.05$). Figure 2 shows the results of evaluating the effect of different treatments on the growth of *L. monocytogenes* bacteria in chicken fillet samples during 15 days of storage at 4°C. According to the results, a significant difference was observed in the count of *L. monocytogenes* in all treatments within 15 days ($P < 0.05$). The control group exhibited the largest increase, with counts rising from 6.01 to 9.03 log CFU/g, representing an increase of approximately 3 log CFU/g. The group treated with gelatin alone recorded an increase of 2.17 log CFU/g ($P < 0.05$). The gelatin coatings containing *L. rhamnus* and *B. bifidum* demonstrated the lowest increases, indicating the most effective inhibition of *L. monocytogenes*, with increases of 1.35 and 1.67 log CFU/g, respectively ($P < 0.05$).

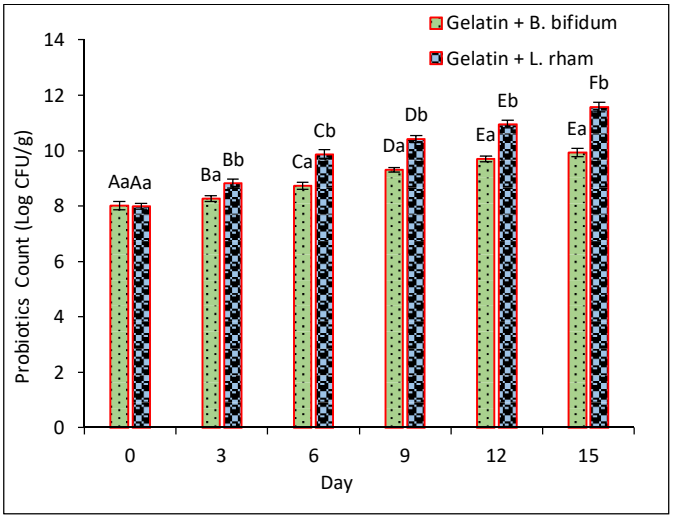

Figure 1. Survival results of *L. rhamnosus* and *B. bifidum* bacteria in gelatin coating during 15 days of storage at 4 °C. Different lowercase letters indicate significant differences between groups and different uppercase letters indicate significant differences within days for each treatment ($P < 0.05$)

The results of the sensory evaluation of chicken fillet samples during 15 days of storage at refrigerator temperature are reported in Figure 3. According to the results, in general, a significant difference was observed in the sensory properties of the preserved samples ($P < 0.05$). In the parameters of aroma, texture, appearance, taste, and overall acceptance, samples packed with gelatin coating containing *L. rhamnus* and *B. bifidum* scored the highest. In the appearance parameter, the highest score was related to the control group, but no significant difference was observed in the samples packaged with gelatin coating ($P > 0.05$). In general, the samples packed with gelatin coatings containing the probiotic bacteria *L. rhamnosus* and *B. bifidum* got the highest score and did not have an adverse effect on their sensory properties, and it was found that this coating caused the organoleptic properties of the product to be preserved. In the current study, the antimicrobial activity of gelatin coating containing *L. rhamnus* and *B. bifidum* was investigated on the growth of *L. monocytogenes* inoculated in chicken fillets during 15 days of storage in the refrigerator. The results confirmed the viability of probiotics at 4°C and showed a significant inhibition of *L. monocytogenes* growth. The findings suggest that gelatin edible coatings can be used

as a suitable matrix for incorporating probiotic bacteria, enhancing food safety of the food and increasing shelf life (Taghizadeh Andevari & Rezaei, 2012). Previous studies have also explored similar interventions. For instance, Taghizadeh Andevari and Rezaei (2012) studied gelatin coatings with cinnamon essential oil on rainbow trout fillets and concluded that total bacterial counts remained within permissible limits until day 10 in both the control and 4% gelatin treatments, while a gelatin-cinnamon combination maintained acceptable levels until day 15. They also reported that gelatin alone was ineffective in reducing the microbial load, but it was effective when combined with cinnamon essential oil. Also, Gómez et al. (2009) reported that gelatin coatings did not exhibit antibacterial properties, while García Argueta et al. (2016) found no significant differences in microbial populations between gelatin-coated and control groups (Gómez-Estaca et al., 2009; García-Argueta et al., 2016).

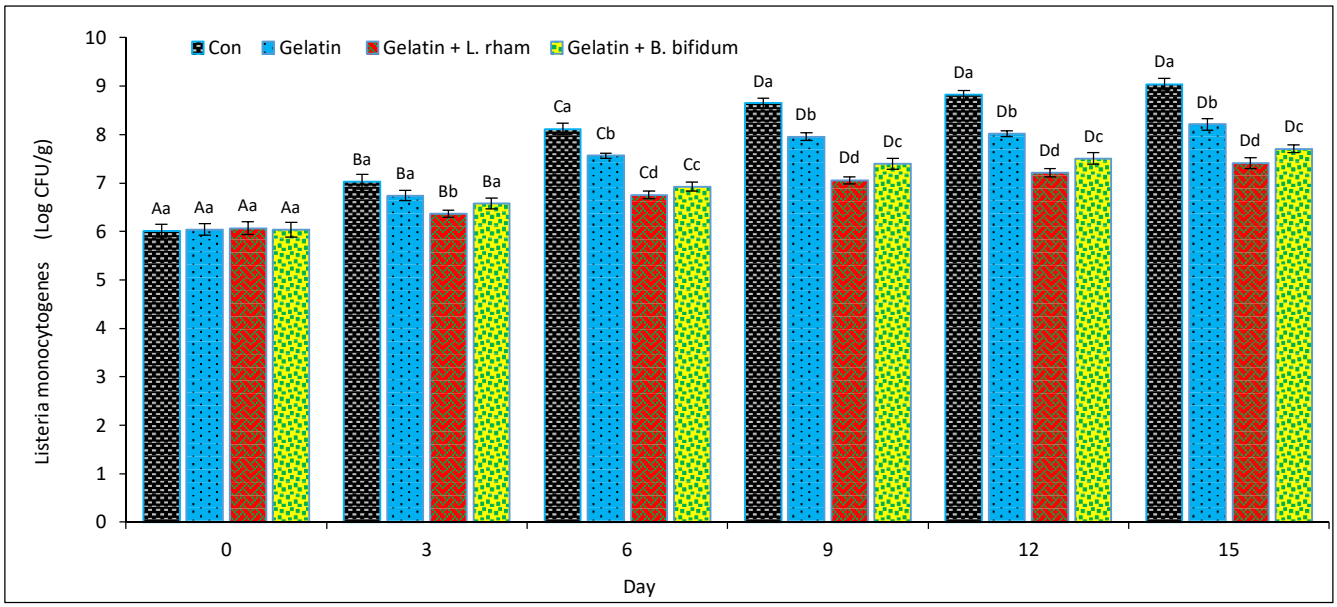

Figure 2. The effect of different treatments on the growth of *L. monocytogenes* bacteria in chicken fillet samples during 15 days of storage at 4 °C. Different lowercase letters indicate significant differences between groups and different uppercase letters indicate significant differences within days for each treatment ($P < 0.05$)

The protective role of gelatin may stem from its ability to form a physical barrier against moisture and oxygen penetration. On the other hand, De Lacey et al. (2012) showed that a coating of 70% gelatin and 30% chitosan reduced bacterial loads in cod meat cakes compared to chicken fillets. A related study of De Lacey et al. (2012), investigated gelatin films containing *Lactobacillus acidophilus* and *B. bifidum* in fish fillets, reporting that both probiotics survived and proliferated, consistent with the present findings. Vermeer et al. (2004) indicated that lactic acid bacteria could significantly reduce *L. monocytogenes* in sausage after eight weeks of refrigeration, suggesting that probiotic treatments were more effective than those used in this study. (Vermeiren et al., 2004). Maragkodakis et al. (2009) identified various lactic acid bacteria as protective cultures against *L. monocytogenes* and other pathogens in chicken, further supporting the potential of probiotic applications. In this study, 635 lactic acid bacteria with nutritional origin were evaluated for their protective effects in chicken. The two-way inhibitory action of these bacteria was visible in laboratory conditions against gram-positive bacteria of the index, mainly *L. monocytogenes* and *Broccocratic* species, while no antibacterial activity against none of the tested gram-negative bacteria was observed.

Specifically, strains of *Enterococcus fasium* (PCD67) and *Lactobacillus* (DC74-ACA) were selected for use in raw chickens as protective cultivation against *Listeria monocytonic* and *salmonella*, with significant growth inhibition noted, particularly against *salmonella*. These findings align with those of Maragkoudakis et al. (2009) which highlighted the efficacy of lactic acid bacteria as protective cultures (Gialamas et al., 2010; Kailasapathy & Chin, 2000; Vanaki et al., 2024). Numerous studies indicate that many strains of lactic acid bacteria serve as effective protective cultures (Kailasapathy & Chin, 2000; Vanaki et al., 2024). In the present study, *Lactobacillus rhamnosus* and *B.bifidum* were incorporated into gelatin coatings for this purpose. Abdollahzadeh et al. (2018) examined sodium caseinate films containing *Lactobacillus acidophilus* and *Lactobacillus casei* as controls against *L. monocytogenes* in fish fillets, finding that the number of both probiotic bacteria increased while the count of *Listeria* decreased, which supports the findings of the current study. Guerrieri et al. (2009) investigated the survival and proliferation of *L. monocytogenes* in biofilms containing lactic acid bacteria, reporting that *Lactobacillus plantarum* achieved the greatest reduction in *Listeria* counts by day 14, with a notable reduction of 1.3 logarithmic cycles during the first two days

of incubation. This reduction was attributed to the production of bacteriocins, a decrease in pH due to lactic acid production, and competitive exclusion of *Listeria*. The results of the present study further confirm that gelatin coatings containing *L. rhamnosus* and *B. bifidum* significantly inhibited the growth of *L. monocytogenes*, reinforcing the potential of probiotic-enhanced edible coatings in food safety applications.

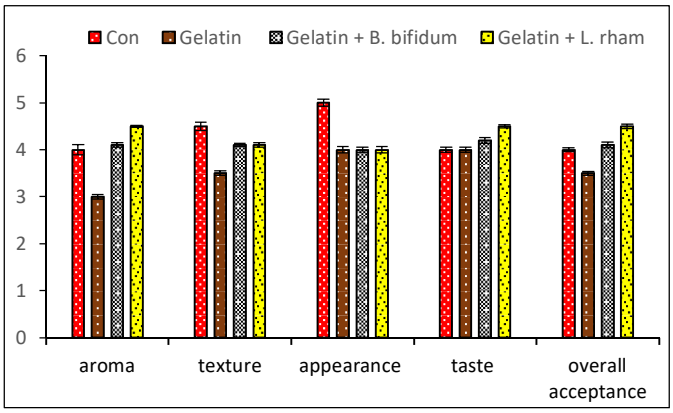

Figure 3. The effect of different treatments on the sensory characteristics of chicken fillet during 15 days of storage at 4 ± 1°C

4. Conclusion

In general, the results of this study showed that the use of this type of food packaging (food packaging containing probiotic bacteria) as an active packaging can effectively control the growth of *L. monocytogenes* pathogen in chicken fillets. Therefore, gelatin edible coating with the properties of adhesion, crystallization, shaping, emulsification, and water absorption can act as a suitable matrix for a combination of probiotic bacteria in some foods to control pathogens.

Authors' Contributions

Mehrdad Rezaeian: Investigation; Methodology; Writing the original draft. **Saeid Hadi:** Methodology; Resources; Visualization; Writing-review & editing. **Vahid Hadi:** Data curation; Software; Formal analysis; Validation. **Asghar Azizian:** Supervision; Project administration; Funding acquisition; Validation; Writing review & editing; Data curation; Software; Formal analysis; Validation.

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Conflicts of Interest

There are no conflicts of interest to declare.

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Ethical considerations

This study was approved by the Ethics Committee of the AJA University of Medical Sciences (Code 1400/262). According to this code, the authors declare that all stages of the study were conducted in compliance with human ethical principles.

Using artificial intelligence

Any type of artificial intelligence was not used.

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