The anti-staphylococcal activity of probiotic-contain gelatin and whey coatings on processed chicken breast

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Abstract

In the current study, processed-cooked chicken breast has been covered by edible coats of whey protein concentrate and gelatin containing *Lactobacillusplantarum* and *Bifidobacteriumbifidum* bacteria. Then, to evaluate the anti-staphylococcal activity of the coatings, the samples were contaminated with *Staphylococcus aureus* (10^5CFU/g), and the population of *S. aureus* was counted in the treated samples on the 1st, 15th, 30th, and 45th days of the storage period by surface culture method. Data were analyzed for statistical significance by analysis of variance (ANOVA) and the Kruskal-Wallis test. Generally, *S. aureus* growth has increased with increasing the time on all treatments and control. However, coated samples with gelatin coats containing probiotics showed more anti-staphylococcal activity than control samples on days 1 and 15. Regarding the samples coated with whey protein, of course, on days 1 and 15 and only in the samples containing *L. plantarum*, the anti-staphylococcal effect was significant compared to the control. (p<05). While there was no difference in the antimicrobial activity of the types of coatings (gelatin/whey) containing *L. plantarum* on all test days (p>0.05), the inhibitory effect by the gelatin coating in the presence of *B. bifidum* was significantly higher on the 15th day (p<0.05). Accordingly, it seems that using probiotics in edible coats may be a hopeful way to cover types of meat products, especially cooked processed meats.

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1. Introduction

Nowadays, poultry production's high and determinant role in supplying animal protein to society has been revealed more than ever [1][2]. Generally, chicken is one of human's most demanded protein sources and is widely consumed in many countries in recent decades. Unfortunately, despite having proteins with high digestibility, desirable taste, and low calories, it is known as one of the potential sources of food-borne pathogens for humans [3][4]. Indeed, chicken meat is a very perishable food that provides a relatively desired environment for bacteria growth, including bacteria causing spoilage and pathogenic [5][6]. In this respect, one of the primary public health challenges is food contamination of animal origin and especially poultry by various pathogens such as *Salmonella* spp.*, Campylobacter, Staphylococcus aureus, Escherichia coli,* and *Listeria monocytogenes,* which, in addition to the irreparable damage it causes to people's health, also has many economic consequences [7][8][9][10]. *S. aureus* is one of the most important pathogenic bacteria in humans and animals [11], which causes food poisoning due to the production of heat-resistant enterotoxin in food contaminated with bacteria [12][13]. Usually, severe reactions such as diarrhea, nausea, and vomiting occur in persons after a few hours of consuming contaminated food. Creamy pastries, meat, poultry meat products, and eggs are the common carriers of this bacteria [14].

According to the harmful effects of chemical antimicrobials, using natural compounds such as essential oils and plant extracts or their derivatives in edible coatings and films has notably increased to enhance the shelf life of food products [15][16][17]. These are a thin layer of edible materials used as the cover on the surface of foodstuffs[18]. Immersion, spray, foam, dribble, and brushing are among the methods to create coats, and the immersion method is the most common. It is should be noted that, using the proper types of film ingredients based on the type of food and expected properties is a principal step in operating the proper functioning of films/coats [19].

Today, gelatin is used for various purposes in the food industry. Due to preventing water and oxygen penetration, it is considered a suitable choice for film and coating formulation to protect meat products [20][21]. More prescient, it delays dehydration and oxidation of myoglobin and lipid and increases the self-life of products [20]. In addition, whey protein has been introduced as one of the proteins that can be used in coatings and edible films, especially for protecting food sensitive to moisture and oxygen, and recently attracted the attention of many researchers in the world [22]. Biological protection is also one of the most up-to-date and popular techniques of food preservation, the effectiveness of which depends on various factors such as acid-lactic bacteria strains, bacteriocins, bacteriophages, etc. [17][23]. Various strains of *Lactobacillus* and *Bifidobacterium* are the most common probiotics used in food, although Enterococcus and *Pediococcus* are also used [24]. Interestingly, their survival and protective activity improve when used in the coatings and films, which is very promising for the food industry promotion [25].

According to our best knowledge, controlled amounts of natural antimicrobials and antioxidants in package films could significantly increase shelf-life, safety, and quality of food. Regarding the high per capita consumption of chicken meat and its vulnerability to microbial and chemical spoilage, the present study has investigated the anti-staphylococcal effect of whey protein concentrate and gelatin edible coats containing *L. plantarum* and *B. bifidum* on the processed-cooked chicken breast, during cold storage (4°C) time.

2. Materials and methods

2.1. *Materials and Bacteria strain*

All materials and cultural media used in the current study belonged to Merck, Germany. Whey protein concentrate was of Hilmar, Made in the USA. The cooked-processed chicken breast was purchased from Andre Meat Products Company in Iran. The strains of *L. plantarum* 1058 (ATCC8014) and *B. bifidum* 1644 (DSM20456) probiotic bacteria have been purchased lyophilized from Iranian Research Organization for Science and Technology. *S. aureus* (ATCC 6538) was obtained from the microbiology laboratory of the Faculty of Health (Qazvin University of Medical sciences, Qazvin, Iran).

2.2. *Preparing probiotic bacteria*

L. plantarum and *B. bifidum* were inoculated in 10 ml Man, Rogosa, and Sharpe (MRS; Merck, Germany) broth and incubated at 37°C for 24 hours under anaerobic conditions. The cultures were aseptically transferred to 95 ml MRS broth and incubated under previous conditions. Finally, the cell suspensions were centrifuged at 1500×g for 15 min at RT and washed twice with 0.1 % sterile peptone water [26]. In the following, the freshly prepared bacteria sediment was used in the coat preparation.

2.3. *Formulation and coat solution preparation*

Coat solutions of whey protein concentrate were prepared for each probiotic by solving 10% (w/v) of it in deionized water. Then, 5% glycerin (w/w) was added as a plasticizer. The solutions were completely homogenized for 2 hours on a magnetic stirrer to ensure an even dispersion. The solutions were then placed in a water bath at 80°C for 20 min to kill potential pathogens and next were cooled to reach the RT. The gelatin coat solution was prepared by mixing 3 gr of gelatin powder in 100 ml of distilled water; Next, glycerin with 25% concentration was added to the above solution and stirred at 45°C for 10 min. Probiotic strains under study (equal to 10^9 CFU/ml) were separately added to the coat solutions.

2.4. *Coating Chicken Breast Samples with the solutions*

Cooked-processed chicken breast samples were immersed for 2 min in each of the solutions. After covering the entire surface of the chicken breast, excess liquid was washed (30 s) from the sample surface. The coated chicken breast samples were deliberately contaminated with *S. aureus* (10^5CFU/g) and then, packaged in vacuum conditions. The samples were transferred to the refrigerator and kept for 45 days. On days 1, 15, 30 and 45, *S. aureus* population was counted in each of the samples by surface culture on specific culture media [27].

Generally, the samples were analyzed in five different conditions, including the following items: 1- cooked, processed chicken breast (uncoated), 2- chicken breast coated with whey protein coat solution containing *L. plantarum* strain, 3- chicken breast coated with whey protein coat solution containing *B. bifidum* strain, 4- chicken breast coated with gelatin coat solution containing *L. plantarum* strain, and 5- chicken breast coated with gelatin coat solution containing*B. bifidum* strain [27].

2.5. *Statistical analysis*

First, data were collected from three repetitions of the treatments. One-Way ANOVA and Kruskal Wallis tests (at $p < 0.05$ %) were used to evaluate statistical differences in SPSS software Version 22.

3. Results and Discussion

Table 1 shows the details of the observed results of the growth control of*S. aureus* inoculated on the chicken breast after covering with the probiotic bacteria-containing gelatin/whey coatings. On the 1st and 15th days of the test, the number of *S. aureus* population counted in the coated sample with gelatin coat containing probiotics was significantly ($p<0.05$) less than the control sample; however, no significant difference was recorded on the 30th and 45th days ($p>0.05$), and the growth of *S. aureus* has increased with increasing the time on all treatments and control (Figure 1). In addition, gelatin coating with variable probiotics (*L. plantarum* and *B. bifidum*) showed similar inhibitory activity against *S. aureus* inoculated into the chicken breast. Regarding the samples coated with whey protein, of course, on days 1 and 15 and only in the samples containing *L. plantarum*, the anti-staphylococcal effect was significant compared to the control. ($p<05$), and the whey coating containing *B. bifidum* probiotic did not show significant inhibition ($p>05$). In general, there was no difference in the antimicrobial activity of the types of coatings (gelatin/whey) containing *L. plantarum* on all test days ($p>0.05$), but in the case of *B. bifidum*, the inhibitory effect under the gelatin coating was significantly higher on the 15th day ($p<0.05$). Specifically, the protection effect of edible gelatin coats containing probiotics was higher than two other treatments during storage. However, comparing treatments and controls shows that both probiotic-content coatings significantly reduced *S. aureus* growth. In this regard, the study of the effect of edible coating of whey protein isolate containing Lysozyme on the microbial quality of chicken fillet kept in the refrigerator showed a significant difference between the microbial count of samples coated with whey protein containing Lysozyme and the control group; In addition, an increase in antimicrobial activity depending on the concentration of the enzyme was reported in the mentioned study, which confirms the results of the present study and shows that the antimicrobial activity of whey coating can be affected by accompanying factors [28]. In another study, no significant growth of Enterobacteriaceae and*S.aureus* has been reported in sliced ham covered with whey protein edible coat containing *L.casei-01* or *B.animalis Bb-12*, which, similar to the present study, confirmed the importance of coatings in providing food safety. The observed inhibitory activity may be related to the competition mechanism of probiotic bacteria with pathogenic/spoilage organisms or the production of antimicrobial metabolites of probiotic strains such as lactic acid or bacteriocins [29][27]. The observed difference in the anti-staphylococcal impact of Bifidobacterium with the current study may also be caused by the difference in the type of bacterial strain or the effect of the whey protein coating. However, Taghizadeh *et al.* showed no significant difference in total count of bacterial and cryophilic bacteria in the fillets coated by gelatin and control group [21]. Besides, Gomez-Estaca *et al.* stated that gelatin film alone (or with chitosan) did not have antimicrobial activity in raw sliced salmon preservation, but its incorporation with clove essential oil showed a significant antimicrobial effect [30]. Although in another study, a film consisting of 30% chitosan and 70% gelatin notably reduced bacterial count in fish patties [31].

Table 1. The count of *S. aureus* (log CFU/ml) in the samples during 45 days of storage at 4°C.

Time(day)	Treatment				
	W1	W2	G1	G2	C
1	$1.2\times10^{6}\pm1\times10^{6}$ b	$1.5\times10^{6}\pm1\times10^{6}$ ab	$1.2\times10^{6}\pm1\times10^{6}$ b	$1.2\times10^{6}\pm1\times10^{6}$ b	$4\times10^{6}\pm1\times10^{6}$ a
15	$2.5\times10^{6}\pm1\times10^{6}$ bc	$4.93\times10^{6}\pm2.08\times10^{6}$ ab	$1.7\times10^{6}\pm1\times10^{6}$ c	$1.7\times10^{6}\pm1\times10^{6}$ c	$5.30\times10^{6}\pm1\times10^{6}$ a
30	$7.8\times10^{6}\pm1\times10^{6}$ a	$6.7\times10^{6}\pm1\times10^{6}$ a	$5.3\times10^{6}\pm1\times10^{6}$ a	$5.3\times10^{6}\pm1\times10^{6}$ a	$6.90\times10^{6}\pm1\times10^{6}$ a
45	$9.7\times10^{6}\pm1\times10^{6}$ a	$8.9\times10^{6}\pm1\times10^{6}$ a	$8.1\times10^{6}\pm1\times10^{6}$ a	$8.1\times10^{6}\pm1\times10^{6}$ a	$7.80\times10^{6}\pm1\times10^{6}$ a

C: control samples. W1: samples with whey protein edible coat containing *L. plantarum*. W2: samples with whey protein edible coat containing *B. bifidum*. G1: samples with edible gelatin coat containing *L. plantarum*. G2: samples with edible gelatin coat containing *B. bifidum*. The letters in each row shows the significant level ($p<0.05$) of comparison between groups; The groups with the same letters are not statistically significant.

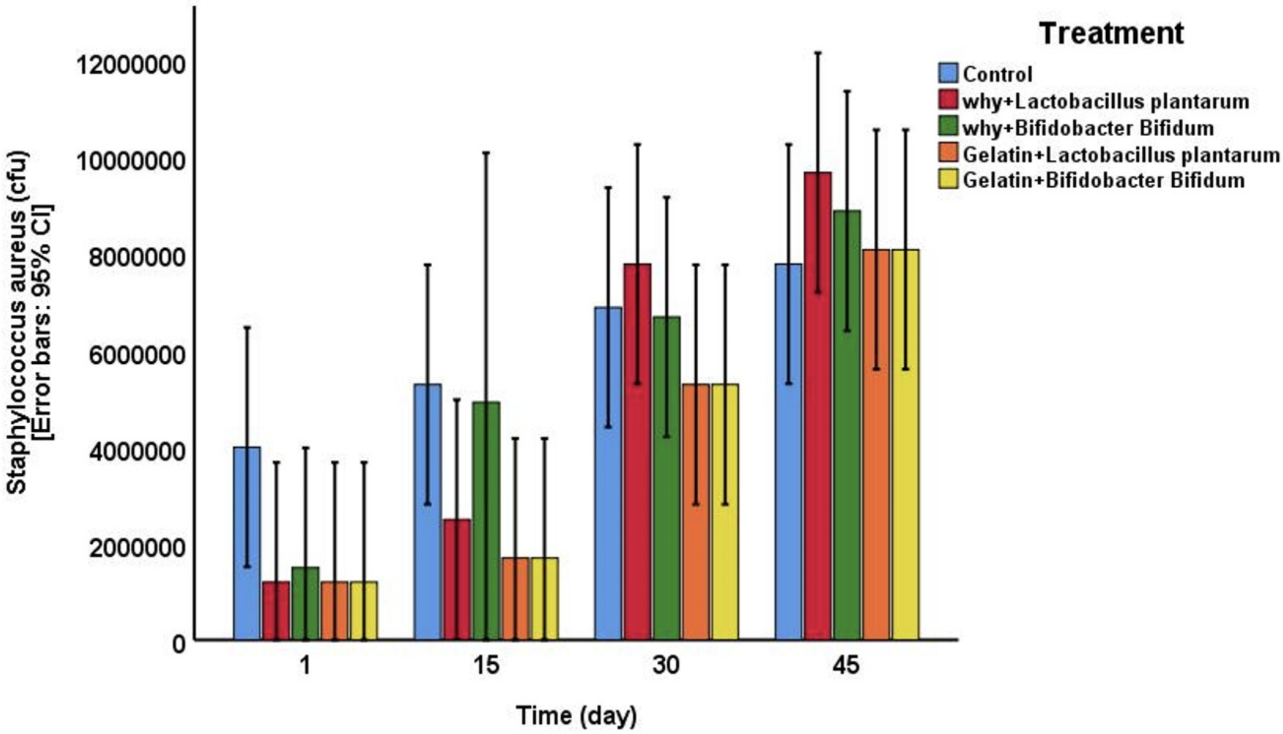

Figure 1. *S. aureus* population during the cold storage period in coated chicken meat with edible coatings.

In addition to gelatin and whey protein discussed in this study, various other coatings have been investigated in food protection. For instance, Juck *et al.* [32] and Neetoo *et al.* [33], in two different studies, investigated the anti-Listeria effect of some coating such as pectin, alginate, carrageenan, xanthan, and starch with antimicrobial compounds including nisin, sodium lactate, and sodium diacetate on boiled-fried turkey or cold-smoked salmon slices and fillets; the results showed that alginate incorporated with any antimicrobial compound was more effective than other coatings. Based on the authors' argument, it could be due to the proper interactions of antimicrobial compounds with alginate and the controlled release of these compounds during preservation [33]. In confirmation of the effectiveness of alginate-based coatings, the results of another study showed that the growth of indicator bacteria, including *S. enteritidis*, *E.coli*, *L.monocytogenes*, and *S. aureus*, was significantly controlled in raw chicken meat coated by alginate coating containing pomegranate peel extract [34].

The study of Fernandez *et al.* showed that antimicrobial-free edible coating might not be effective in controlling the microbial load; in fact, there was no significant difference between microbial count in chicken meat with and without a coat in the absence of antimicrobial compounds; Their study also revealed that using antimicrobial compounds inside edible coating is more effective than using them in free form [18] . Generally, antimicrobial compounds existing in the edible films and coatings matrix are released in a controlled manner, which increases its effectiveness.

As far as we know, Lactic acid bacteria, as an important antibacterial, are most effective in human health during consumption, they also create a protective effect against pathogenic microorganisms in food products during storage by competing with pathogenic factors on nutrients and producing metabolites like organic acids and bacteriocins. The production of lactic acid has a noteworthy effect on limiting the growth of pathogenic bacteria in foodstuffs, probably due to hydrogen-effective ion leakage through the cell membrane. Researchers have shown that the effect of antimicrobial metabolites, like other antimicrobials, in combination with coatings and films is more than their free form. This advantage of combining bacteria in films and coatings may be related to the expansion and concentration of bacteria at the surface of the product. More precisely, it may be due to the reduction of release from the film/coat to the product, in which a high concentration of the active compound is preserved at the surface of the product and protects food against pathogenic or spoilage organisms [35].

4. Conclusion

In general, the findings of the present study showed the effective inhibition of *S. aureus* during the storage period in coated chicken meat with edible coatings such as probiotics-containing whey protein concentrate and gelatin compared to uncoated samples. Accordingly, it seems that using probiotics in edible coats may be a hopeful way to cover types of meat products, especially cooked processed meats. Indeed, antimicrobial packaging is an innovative approach in an active packaging concept that could increase shelf-life and improve safety and even properties because of their desired interaction with foodstuffs. However, comprehensive and extensive studies on different types of sensitive food should be designed and carried out to achieve the correct attitude about the effectiveness of edible films and coatings containing probiotics.

Statements and Declarations

Conflicts of Interest

The authors declare that they have no conflict of Interest.

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