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Study on The Impact of Gelatin Coating Containing *Lactobacillus acidophilus* and *Lactobacillus reuteri* on Chicken Fillets Shelf Life

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ABSTRACT: The purpose of this study is to assess how chicken fillet shelf life and sensory evaluation are affected by 1% Gelatin coating containing *Lactobacillus acidophilus* PTCC 1643 and *Lactobacillus reuteri* ATCC 1655. Probiotic-containing cultures were introduced straight to the coating solution in this investigation. Measures were taken of the chemical (pH, TVB-N, peroxide value), microbiological (Total viable count (TVC), psychrotrophic count (PTC), *Enterobacteriaceae*, and *Pseudomonas* spp.), and sensory properties. During the storage of the chicken fillet, which was kept at 4° C for intervals of 0, 3, 6, 9, and 12 days, the antimicrobial properties of the coating in the groups treated with gelatin, *Lactobacillus acidophilus*, and *Lactobacillus reuteri* were significantly (p<0.05) higher than those in the control groups, despite the fact that the results showed the highest chemical properties and sensory score across all parameters. However, gelatin + *Lactobacillus reuteri* and gelatin + *Lactobacillus acidophilus* did not differ significantly (p<0.05). Additionally, samples treated with gelatin and probiotics showed no changes in color or texture, but their odor and taste scores decreased. We draw the conclusion that gelatin is an appropriate matrix for probiotic incorporation and long-term fillet storage.

Keywords: edible coating, gelatin, *L. acidophilus*, *L. reuteri*, chicken fillets

INTRODUCTION

In the most recent decade, the incomparable properties of edible films and coatings have been extensively studied. On the other hand, film and coating can incorporate microbial culture, and the adequate amounts of fused probiotic microscopic organisms in film and coating enable them to arrive at the consumer's gut to give medical advantages to the host. Food shelf life can be increased by adding bacteria or yeasts to the films, which can be controlled biologically (Guimaráes *et al*., 2018).

Comprising of partially hydrolyzed collagen, gelatin is a soluble protein that has been linked to antibacterial activities and cell reinforcement. The skin, ligaments, and bones are the main sources of gelatin. The internal factors, such as collagen type, age, and source, influence the characteristics of gelatin (Gómez *et al*., 2011). Maintaining the gelatin structure is essential for food packaging applications in order to ensure the stability of the film in humid environments. Until date, glutaraldehyde has been employed as a cross-linker agent; however, because it is a systemic and cell-toxic molecule, it has a significant drawback (Biscarat, J. *et al*., 2015).

According to the definition given by the World Health Organization and Food and Agriculture Organization of the United Nations (FAO/WHO) in 2002, probiotics are "live organisms that, when consumed in adequate amounts, confer beneficial effects on the host" (Pavli *et al*., 2018). There are a couple of instruments by which probiotics may benefit humans, including the production of antimicrobial material, strengthening of the intestinal barrier, adjustment of the immune response, also threat of pathogenic microorganisms either by the creation of antimicrobial operators or by rivalry for restricting locales, supplements, and development factors (Parvez *et al*., 2006). Conversely, lactic acid bacteria (LAB) are given taste and surface and increment the healthy benefit of matured meals, for example, yogurt, aged cheddar, and meat items, as well as certain vegetables. A lot of research has concentrated on LAB for sustenance bio-protection, including bacitracin-creating strains (Concha *et al*., 2011). As well as the *Lactobacillus reuteri* is a built-up probiotic operator, the most broadly circulated *Lactobacillus* species among creatures, and is viewed as one of a predetermined number of indigenous *Lactobacillus* species in the human digestive tract. Also, an essential antimicrobial compound created by *L. reuteri* is reuterin, created during glycerol maturation. A b-hydroxypropionaldehyde (3-HPA), a subordinate of glycerol, is created under anaerobic conditions and

displays an expansive range of impacts against microscopic organisms that are both gram-positive and gram-negative (Spinler *et al*., 2008).

As well as species *L. acidophilus* has been broadly examined and ascribed with qualities, for example, advancing a helpful tweak of the metabolic action of intestinal microbes, which can avoid diarrhea related to antibiotic use (Ouwehand *et al*., 2014). It also safeguards intestinal respectability during radiotherapy, animating the immune response, and stimulating the creation of lactase, which helps in the processing of lactose and improves intestinal microflora (Demers *et al*., 2014).

Meat and special chicken fillets are consumed all over the world by consumers. However, quick spoilage is one of the biggest problems. Therefore, nowadays, food producers are looking for innovations to expand the shelf life of fillets (Petrou *et al*., 2012). Meat has been shown to be an effective carrier for probiotics. The limited buffering capacity of meat may be due to the elevated pH of the microenvironment where microorganisms live. Additionally, meat has been found to protect probiotics from the harmful effects of bile (Rivera *et al*., 2010).

Tapia *et al*. first suggested the addition of probiotics to this edible process (2007). In addition, *L. acidophilus* and *L. reuteri* have joined forces used in Sodium caseinate and methylcellulose films with glycerol showed themselves to be an efficient vehicle of these bacterial cells, utilized as antimicrobial operators, also the films with bioactive microbial load presented interesting activity against listeria (Sánchez *et al*., 2014). Otherwise, food preparation conditions, for example, heat, osmotic, or mechanical pressure, can reduce the livability of probiotic culture. To conquer these issues, a few techniques have been proposed throughout the most recent years, the use of edible films as a transporter for probiotic cells in a food bioactive packaging system can deliver a medicinal advantage to consumers and greater survivability (Espitia *et al*., 2016; Soukoulis *et al*., 2017).

Gelatin is a good foundation for probiotic collecting, according earlier research. Numerous studies on the impact of bacteria on probiotics or antibiotics for this film and procedure have been published in the previous ten years. However, no studies have been performed on gelatin coatings containing *L. acidophilus* and *L. reuteri* on chicken fillets. This arrangement can help improve the supply of fresh and healthy chicken fillets with the least biodegradation.

MATERIALS AND METHODS

Bacterial strains

Lactobacillus acidophilus (PTCC 1643), *Lactobacillus reuteri* (ATCC 1655) were acquired from the Scientific and Technology Research Organization of Iran.

Creating the Coating Solution Formula

Amstel Netherlands gelatin was used to make the coating-forming solutions. Both glycerol (0.30g/g gelatin) and gelatin1%, which was prepared by dissolving 1g/100ml of distilled water, were utilized as plasticizers. To create bioactive packaging, the strains of *Lactobacillus* acidophilus PTCC 1643 and *Lactobacillus reuteri* ATCC 1655 were chosen. To get a final concentration of 109 cfu/ml, *L. acidophilus* and *L. reuteri* were added to their respective coating solutions. Consequently, a total of five samples were obtained:

(C) Control solution without gelatin and probiotics
(G) Gelatin 1%
(G + La) Gelatin 1% + *L. acidophilus*
(G + Lr) Gelatin 1% + *L. reuteri*
(G + La + Lr) Gelatin 1% + *L. acidophilus* + *L. reuteri.*

Chicken storage trail

Chicken fillets were bought from a local market near the University of Tehran's veterinary medical faculty, and transferred in cold conditions to the laboratory. The fillets were divided according to the need of the test 40g of the fillet samples for chemical and sensory evaluation tests and 10g for microbial evaluation of fillets were prepared in a sterile condition, a total of 50 fillets were weighted for each treatment. The portions were dipped into the solution (with or without gelatin and probiotics) for 20 minutes and then dried on the sterile metal lace then collected and stored in sterile containers at 4 °C until the next analysis at different days of intervals (days 0, 3, 6, 9, and 12).

Bacteriological examination

10g of prepared samples were combined with 90 ml of 0.1% peptone water, mixed in a sterile bag, and homogenized using a Stomacher (Bag Mixer 400, Interscience, France) at 200 rpm/min for three minutes in order to assess the bacterial counts. From this dilution, more decimal dilutions were made in tubes with peptone water. Bacterial counts were performed using a method using plate count agar (PCA) for Total viable count (TVC) and psychrotrophic count (PTC), pseudomonas Agar for *Pseudomonas* spp, and Violet Red Bile Glucose Agar (VRBGA) for *Enterobacteriacea*e. The inoculation

plates were incubated for 10 days at 7 °C for the psychrotrophic count (PTC) and 37 °C for the Total viable count (TVC), *Pseudomonas* spp., and *Enterobacteriaceae*. The colony-forming units per gram (log cfu/g) of a sample was used to express all microbiological counts, and analyses were carried out in duplicate (Jouki *et al*., 2014; Golstani, R *et al*., 2017).

Chemical examination

Measuring pH

To measure the pH with a pH meter, homogenize 5g of chicken fillet sample in 45 ml of distilled water. For every treatment, duplicates of every test were run.

Total Volatile Nitrogen Bases (TVB-N) Measurement

The measurement of TVB-N in chicken different treatment samples examined according to Jouki *et al*. (2014)

Measuring of peroxide value

The peroxide value was determined using the Estaca *et al*. (2007) technique.

Sensory examination

Based on the experience of analysis, a group of 6 education experts were selected from the staff of Tehran University. Samples of chicken were prepared in a microwave oven at 700 watts for 4 minutes. Sensory evaluation is based on a five recognition points of odor and taste and uses recognition to determine a scale from approximately 5 to 0, where 5 corresponds to the most preferred model and 0 to the least preferred model. A score of 3.5 is considered a lower level of acceptance (Chouliara *et al*,. 2007).

Statistical examination

Version 23 of the SPSS, Inc. program was used to analyze the data.

RESULTS AND DISCUSSION

Bacteriological analysis

Figure 1, a, b, c, d, shows the results of the total count (TVC), psychotropic count (PTC), *Enterobacteriaceae* and *Pseudomonas* genus analyses used to evaluate the microbiological the products'quality during storage. In general, the average bacterial count of all five groups showed an increase and the total count (TVC) of chicken fillets with and without probiotics for 12 days of treatment storage at 4 °C is shown in Figure 1a. The predominant bacterium in chicken fillets are as follows (Figure 1). 1. a) 4.97 log CFU/g indicates that the chickens used in this study were good. According to the ICMSF statement in 1986 (Mexis *et al*., 2012), the acceptable level of fresh meat is less than 7 log CFU/g; if the average of the bacteria reaches this level, the spoilage of meat starts. In our study, the load of total viable count in control, G + La, G + Lr was lower in this limit to the 9 days, but in the G + La + Lr was lower than 7 log CFU/g in the 12 days of the storage. The mean of Total viable count load in the whole period was increased in all groups, and the highest rate of Total viable count load on the 12th day of the storage was respectively (Figure. 1. a) 7.58±0.07 related to the control sample, as well as the lowest rate of Total viable count (TVC) load was respectively (Figure. 1. a) 6.31±0.05 related to G + La + Lr. The result of the total viable count has been reported by López de Lacey *et al*. (2012). The difference in total viable bacteria load can be due to the decrease in pH and production of antimicrobial compounds by *Lactobacillus* (Elizabeth Caplice, 1999). The mean of psychotropic bacterial load throughout the whole period was increased in all groups, and the highest load of psychotropic bacteria was respectively (Figure. 1. b) 7.70±0.05 on the 12 days of the storage related to control and the lowest bacterial rate was respectively (Figure. 1. b) 6.84±0.14 in G + La + Lr. The highest load of *Enterobacteriaceae* on the 12th day was 6.88±0.06 in control (Figure. 1. c), and the lowest was 5.85±0.09 in the G + La + Lr (Figure. 1. c). The mean of *Pseudomonas* spp load through the whole period was increased in all groups and the highest load was respectively 7.94±0.06 (Figure. 1. d) on the 12th day of the storage related to control and the lowest bacterial rate was 6.95±0.07 in G + La + Lr respectively (Figure. 1. d).

The current finding in all groups' show that G + La + Lr antimicrobial properties, and Gomez-Estaca *et al*., (2010) concluded that gelatin coating and film do not have antimicrobial properties. Therefore, based on the findings of this investigation, it can be said that the low resistance of gelatin is only due to the formation of the immune system against the penetration of bacteria. On the other hand, the alginate layer is a good support for *Lactobacillus. acidophilus*. This probiotic system also reduces fungal infections and the number of bacteria in the aerobic system. Our study also showed that the formula probiotic-containing samples reduced the number of aerobic bacteria compared to the gelatin samples, which agrees with the findings of this investigation. However, according to López de Lacey *et al*. (2012), gelatin edible coatings and films might provide good matrices for adding lactic acid bacteria to a variety of meals.

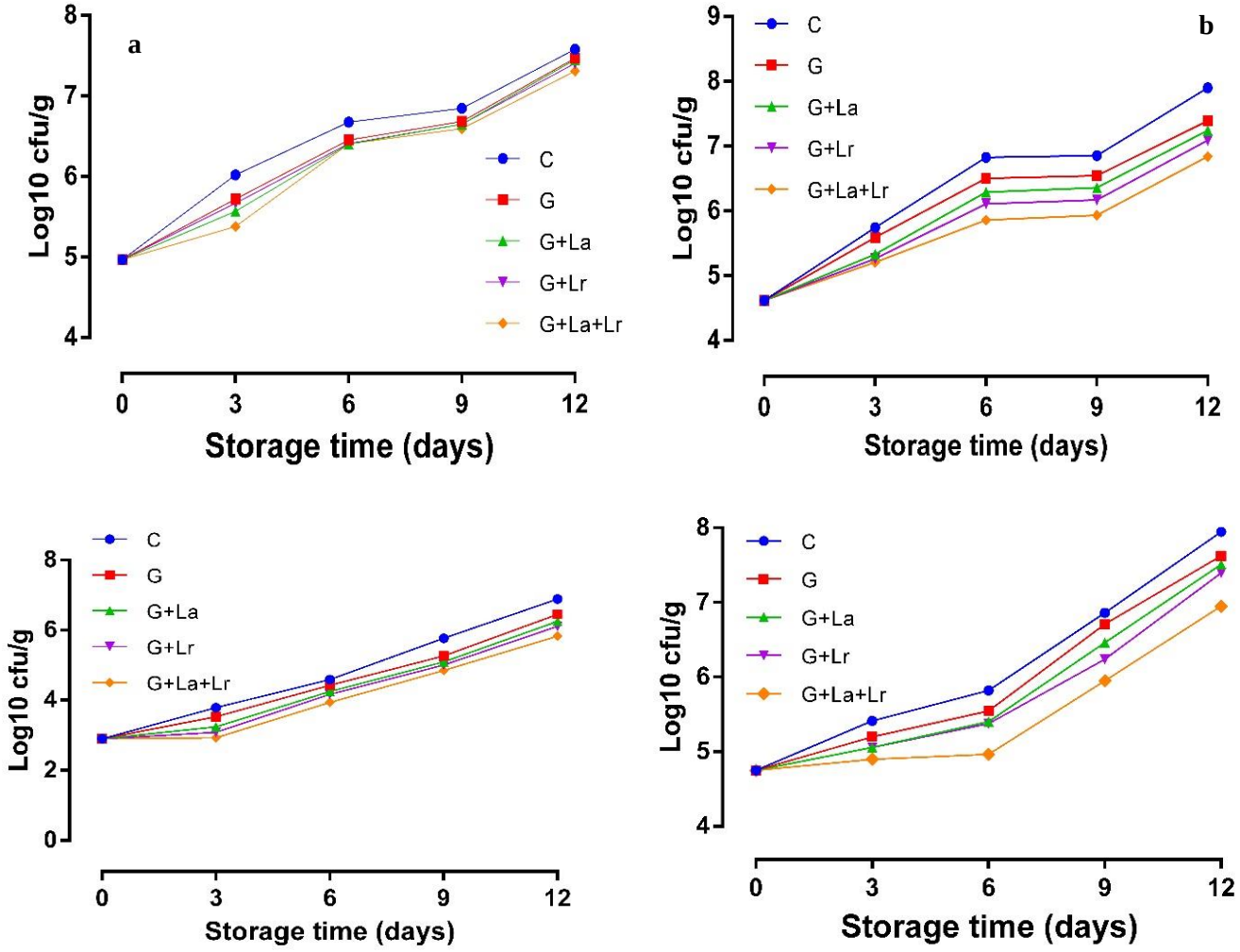

Figure. 1. Total number of viable bacteria (a), *psychotropic* count (PTC) (b), *Enterobacteriaceae* (c), *Pseudomonas* spicies. (d) in chicken fillets dipped without and with probiotics kept for 12 days at 4 °C. C: Control sample without, G: sample dipped with gelatin 1%, G+La: sample dipped with gelatin 1% containing *L. acidophilus*, G+Lr: sample dipped with gelatin 1% containing *L. reuteri*, G+La+Lr: sample dipped with gelatin 1% containing *L. acidophilus* + *L. reuteri.*

Chemical examination

pH

Figure 2 illustrates how the pH of chicken fillets changed while they were being stored. All of the chicken samples had an initial pH of 6.16 before being stored. The control groups and gelatin's pH values grew by 1%, while the pH values of the gelatin + probiotics initially decreased and then increased. López de Lacey *et al*. (2012) noted similar findings, stating that the fish's pH is almost 7. Furthermore, the G + La + Lr sample's pH values were discovered to be lower (6.15) than they had been at the conclusion of the investigation (Figure 2). Otherwise, the presence of lactic acid in the layer, particularly in G+La+Lr, causes a modest decrease in pH. Similar findings were reported by Faan *et al*. (2008), where the sample's pH initially declined and subsequently increased to 6.2, although Lopez de Lacy *et al*. (2012) reported a pH value that was almost seven.

These findings could be attributed to the microbial load's activation effect, which could lead to protein hydrolysis and the generation of volatile basic nitrogen components influenced by low-temperature biochemical processes. It is important to understand that, according to Shah's 2007 report, lactic acid bacteria's synthesis of lactic acid and organic acids has the impact of reducing pH values. Thus, pH influences the proliferation of microorganisms and, thus the shelf life of meat products (Shah, 2007).

Total volatile base nitrogen (TVB-N)

The most used gaseous technique for determining how much beef has spoiled is the estimation of total volatile base nitrogen, or TVB-N. A common test for identifying

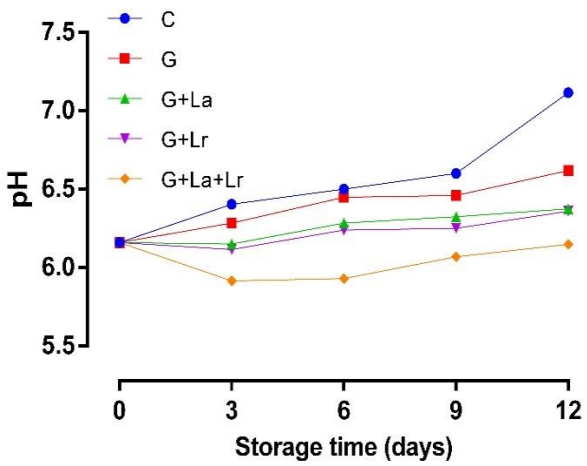

Figure. 2. Changes in pH values in chicken fillets dipped both with and without probiotics while being stored for 12 days at 4 °C. C: undipped control samples, G: sample dipped with gelatin 1%, G+La: sample dipped with gelatin 1% containing *L. acidophilus*, G+Lr: sample dipped with gelatin 1% containing *L. reuteri*, G+La+Lr: sample dipped with gelatin 1% containing *L. acidophilus* + *L. reuteri.*

muscle weakness is the TVB-N, which measures the concentration of primary, secondary, and tertiary amines in addition to ammonia. According to Fan *et al*. (2009), the growth of it is connected to the activities of endogenous chemicals and decay organisms. Figure 3 displays TVB-N readings for each treatment during the storage period.

The fillet sample's initial TVB-N value (8.40 mg TVB-N/100 g; Figure 3) in this investigation was lower than the values proposed by Lopez de Lacey *et al*. (2012). Hake recorded levels of about 10 mg N-TVB/100 g muscle of total volatile basic nitrogen at the start of the study. Additionally, they are higher than those Ranjbar & Azizi (2017) reported for chicken fillets kept in the refrigerator. According to Christiana (2006), an upper tolerance limit for the onset of spoiling for fresh chicken is 28–29 mg N/100g for TVB-N values.

All TVB-N levels in our analysis were below the suggested limit after 9 days of storage, but the G+La+Lr sample did not drop below the limit until the very end of the investigation. Probiotic samples coated in gelatin, uncoated samples, and probiotic-coated samples all achieved the limit in 9 days during the storage period (Figure 3). Both the coated and uncoated samples' TVB-N values grew significantly ($p < 0.05$) over the course of storage; nevertheless, by day 12, there had been a large increase in TVB-N values.

Samples dipped in G+La+Lr had TVBN values of 27.28, which was lower ($p<0.05$) than samples dipped in gelatin and control samples. The antibacterial characteristics of *Lactobacillus* in the current investigation may be responsible for the decreased TVB-N values in the G + La + Lr samples. Additionally, the TVB-N content in samples dipped in G + La and G + Lr was substantially lower ($p < 0.05$) than in samples dipped in gelatin coating. This was caused by the effect of lactic acid bacteria on chicken fillets, and can be explained by either a faster reduction in the bacterial population or a lower capacity of bacteria for oxidative deamination of non-protein nitrogen molecules, or both (Fan *et al*., 2008).

Peroxide value (PV)

Figure 4 shows how coating affects variations in the PV of chicken lipids. Over the course of storage, the PV values of the coated and control samples increased significantly ($p<0.05$); nevertheless, by the conclusion of the storage period (day 12), there were notable differences ($p>0.05$) in the PV between the control respectively

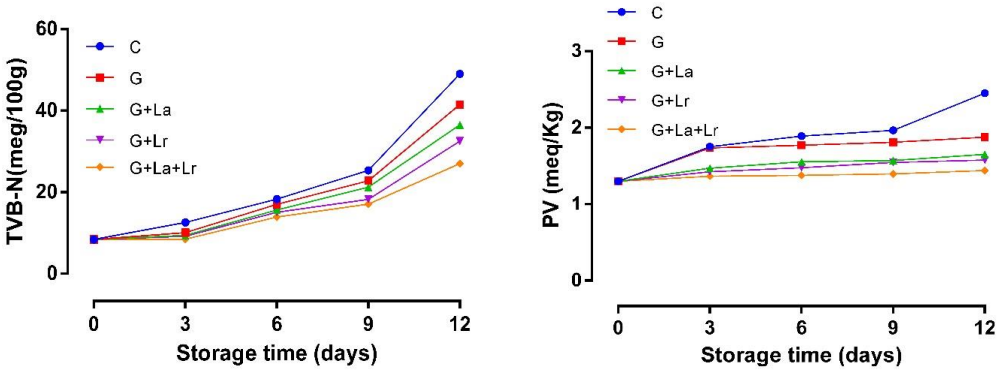

Figure. 3. Changes in TVB-N values in chicken fillets dipped both with and without probiotics while being stored for 12 days at 4 °C. C: undipped control samples, G: sample dipped with gelatin 1%, G+La: sample dipped with gelatin 1% containing *L. acidophilus*, G+Lr: sample dipped with gelatin 1% containing *L. reuteri*, G+La+Lr: sample dipped with gelatin 1% containing *L. acidophilus* + *L. reuteri.*

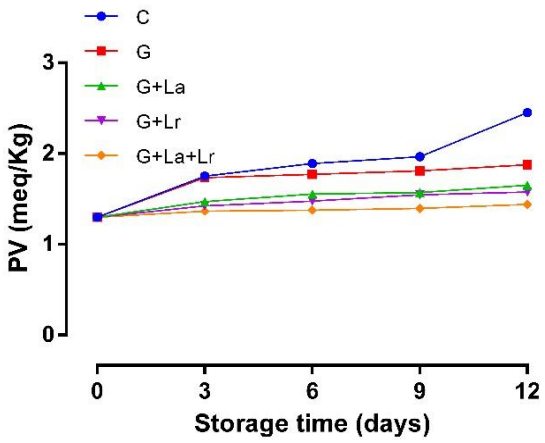

Figure 4. Changes in PV values in chicken fillets dipped both with and without probiotics while being stored for 12 days at 4 °C. C: undipped control samples, G: sample dipped with gelatin 1%, G+La: sample dipped with gelatin 1% containing *L. acidophilus*, G+Lr: sample dipped with gelatin 1% containing *L. reuteri*, G+La+Lr: sample dipped with gelatin 1% containing *L. acidophilus* + *L. reuteri.*

(Figure. 4) 2.45 meq/kg. Gelatin respectively (Figure. 4) 1.88 meq/kg and each of samples coated with G + La, G + Lr, G + La + Lr, exhibiting lower values of 1.65, 1.58, and 1.44 meq/kg respectively (Figure. 4). The current study's findings show that covering chicken fillets with gelatin and probiotics can effectively slow down the creation of PV while keeping them refrigerated at 4 °C. These findings concur with those of Gómez-Estaca *et al*. (2007) and Ranjbar & Azizi (2017), who found that peroxide values in the 0.3% Bene EO treated fillet groups were lower than control. The peroxide index shows lower levels of oxidation in batches covered with films enhanced with oregano or rosemary extract and higher levels in the control batch. Day 0's high readings were caused by smoking, which produces a lot of peroxides, according to Chaijan *et al*. (2005) at the first 10 days of the study indicated oxidation of fat, peroxide value increased significantly, and after 10 days of the study, the PV content was decreased, which could be because of the decomposition of hydroperoxide into products of secondary oxidation. Antoniewski *et al*. (2007) also found that gelatin coating did not significantly reduce lipid oxidation in beef, poultry, salmon, and pork in refrigerator storage. Our study also showed that there are no significant effects observed in gelatin coating.

Sensory Examination

Sensory properties (color, texture, odor, and taste) of cooked chicken fillets are given in Table 1. The reduced level of acceptance score of 3.5 was attained for color, texture, and odor after 6 days for the gelatin and control samples, at the 9 days of the study for the G + La, G + Lr, and G + La + Lr samples. The lower acceptability limit

Table 1. Effect of Gelatin and Probiotics on sensory properties (color, texture, taste, and odor) of coated chicken fillet stored at 4 ℃.

Storage time (day)	Control	Gelatin	Gelatin + La	Gelatin + Lr	Gelatin + La + Lr
Color					
0	4.83±0.40^{aA}	4.83±0.40^{aA}	5.00±0.00^{aA}	5.00±0.00^{aA}	5.00±0.00^{aA}
3	4.50±0.83^{aA}	4.50±0.83^{aAB}	5.00±0.00^{aA}	5.00±0.00^{aA}	5.00±0.00^{aA}
6	3.33±0.81^{bB}	3.83±0.98^{abBC}	4.33±0.51^{aB}	4.33±0.51^{aB}	4.50±0.54^{aA}
9	3.17±0.98^{bB}	3.33±0.98^{bC}	4.00±0.00^{abB}	4.33±0.81^{aB}	4.50±0.54^{aA}
Texture					
0	4.50±0.54^{aA}	4.67±0.51^{aA}	4.83±0.40^{aA}	4.83±0.40^{aA}	5.00±0.00^{aA}
3	4.50±0.54^{aA}	4.50±0.54^{aA}	4.67±0.51^{aA}	4.83±0.40^{aA}	4.83±0.40^{aAB}
6	3.67±0.51^{bB}	4.17±0.75^{abA}	4.50±0.83^{abAB}	4.50±0.54^{abAB}	4.67±0.51^{aAB}
9	3.00±0.89^{cB}	3.33±0.81^{bcB}	3.83±0.40^{abB}	4.17±0.40^{aB}	4.33±0.51^{aB}
Odor					
0	4.00±1.09^{aA}	4.50±0.54^{aA}	4.83±0.40^{aA}	4.67±0.51^{aA}	4.83±0.40^{aA}
3	4.33±0.81^{aA}	4.83±0.40^{aA}	4.67±0. 81^{aAB}	4.67±0.51^{aA}	4.83±0.40^{aA}
6	2.00±0.00^{cB}	2.67±0.51^{cB}	3.83±0.98^{bBC}	4.33±0.81^{abA}	4.67±0.51^{aA}
9	2.00±0.00^{cB}	2.67±0.51^{bcB}	3.00±0.63^{abC}	3.17±0.75^{abB}	3.67±0.81^{aB}
Taste					
0	4.50±0.83^{aA}	4.67±0.51^{aA}	4.83±0.40^{aA}	5.00±0.00^{aA}	5.00±0.00^{aA}
3	4.17±0.98^{bA}	4.50±0.54^{abAB}	4.67±0.51^{abAB}	5.00±0.00^{aA}	5.00±0.00^{aA}
6	3.67±0.51^{aAB}	3.83±0.40^{aB}	4.17±0.40^{aB}	4.17±0.75^{aB}	4.33±0.51^{aB}
9	2.83±0.75^{cB}	3.00±0.89^{bcC}	3.50±0.54^{abcC}	3.83±0.40^{abB}	4.00±0.63^{aB}

Means in rows A through C that have different capital superscript characters are significantly different (P~<0.05). A-C denotes substantially different (P<0.05) values for the same sensory attribute when represented by different tiny superscript letters in the column.

for taste was reached in the 9 days of the storage for control and gelatin samples, as well as the gelatin + probiotics samples which did not demonstrate the lower acceptability limit until the end of the trial. Samples of La + Lr containing gelatin gave a characteristic desirable to all the sensory properties of the chicken fillets, very compatible with cooked chicken flavor. Both odor and taste proved to be equally sensitive sensory properties for chicken meat, as shown by sensory scores in Table 1. Based on sensory scores, it can be stated that gelatin containing La + Lr had the same effect as that of G + La and G + Lr on the shelf-life extension of fresh chicken fillets. Both G + La and G + Lr had the same effect on the sensory quality of the chicken fillet.

The sensory data and the microbiological data did not agree very well. This is extremely regular, considering that it is not the TVC but the specific spoilage bacteria (SSO) that reason spoiling to the product (Chouliara *et al*., 2007). The sensory evaluation's findings demonstrated that adding *Lactobacillus* by itself can result in customer approval. As well as the addition of *Lactobacillus* in pairs, the panel's comments were more acceptable. However, past research has shown that adding high levels of gelatin can cause undesirable changes in the sensory panel's views.

CONCLUSION

The study's findings suggest that gelatin is a suitable framework for lactic acid bacteria consolidation. As well as gelatin coating alone cannot keep chicken filets at the refrigerator temperature. On the other hand, fusing lactic acid microorganisms into edible coating has been a novel innovation that gives medical advantages to the consumer. The incorporation of LAB culture into the coating significantly affected the bacterial and chemical properties, as well as gelatin coating containing bioactive culture played a high sensory impact during refrigerator storage. Likewise, the utilization of *L. acidophilus* and *L. reuteri* was promising for chicken filet safeguarding.

CONFLICT OF INTEREST

There are no conflicts of interest, according to the researcher.

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