Application of Citrus Waste–Based Films containing Lactobacillus sp. And *Bifidobacterium bifidum* as a Substitute for Parchment Paper in Feta Cheese

Asghar Azizian, Negin Noori *, Hassan Gandomi, Ali Misaghi, Ali Khanjari

Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

***Corresponding author:** Negin Noori, Department of Food hygiene and quality control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran Email: nnoori@ut.ac.ir

Abstract

Introduction: The application of active and biodegradable packaging as a sustainable strategy to enhance the safety and shelf life of dairy products, has gained increasing attention. Therefore, the aim of this study was to evaluate the effectiveness of a probiotic film prepared from citrus waste as a substitute for parchment paper for the storage of feta cheese.

Materials & Methods: Treatments including: cheese samples wrapped with parchment paper, film, and films containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Lactobacillus plantarum*. Then the samples were evaluated through microbiological, physicochemical, and sensory analyses.

Results: The initial probiotic counts in the films on the first day were similar, followed by a gradual decrease during storage. At the end of the storage, the viability *of L. plantarum* (5.33 Log cfu/g) was significantly higher than that of *L. acidophilus* (4.90 Log cfu/g) and *B. bifidum* (3.16 Log cfu/g) (P<0.05). In the evaluation of probiotic release into feta cheese, the film containing *L. plantarum* exhibited the highest release level. Probiotic films, particularly those containing *L. plantarum*, exhibited stronger antimicrobial activity. In feta cheese, the population of *S. aureus* in samples packaged with the *L. plantarum* film reached 4.22 Log cfu/g, which was significantly lower than that observed in samples wrapped with parchment paper (6.21 Log cfu/g) at the end of storage (P<0.05). Moreover, this treatment showed the best performance in controlling coliforms and molds. Incorporation of probiotics into the films resulted in reduced water vapor permeability and increased clarity, without causing significant changes in tensile strength or film thickness. Sensory evaluation indicated that cheeses coated with the *L. plantarum*–containing film achieved the highest overall acceptability scores.

Conclusion: citrus waste based probiotic films, especially those containing Lactobacillus sp., can be proposed as an effective and sustainable substitute for parchment paper for the preservation of cheese.

Keywords: Citrus, Feta Cheese, Parchment, Probiotic, Waste

1. Introduction

Feta cheese is one of the oldest and most renowned brined cheeses in the world, with a production history tracing back about 4,000 years to ancient Greece. It is classified as a white, soft cheese that ripe and is stored in brine. A variety of brined cheeses are produced throughout Eastern Europe, the Middle East and the Balkan Peninsula, with feta being the most emblematic. Typically made from sheep's milk, goat's milk, or a blend of both, feta cheese is distinguished by its white color, crumbly and brittle texture, and characteristic salty taste, making it one of the most widely consumed cheeses in everyday diets [1].

Feta cheese is nutritionally dense too. Feta is a good source of calcium, sodium, folic acid, vitamins A, K, B, B6, B12, and a variety of other fat and water soluble vitamins [1]. As good as feta nutrition looks, a few characteristics of feta cheese makes it susceptible to spoilage and growth of pathogenic microorganisms more than compared to other dairy products. Feta cheese has a high pH, strong buffering capacity, higher fat content, and it has a good scale of water activity combined with a dense cheese matrix [1, 2]. Therefore, challenge the cheese industry to fit a solution of microbial spoilage and maintain the quality of the sample during refrigeration, especially in the feta cheese industry.

In the industrial production of Iranian white feta cheese, plastic parchment paper is widely used to cover the cheese's surface. The sheets made out of petroleum based polypropylene are crucial in helping ripening product quality due to moisture and tear resistance, non-curling, and pH buffering to uniformity to cheese mass [3]. After milk coagulation and adding salt, whey separation occurs and a saturated brine layer is formed on the cheese surface. Parchment paper is beneficial in retaining this brine on the cheese surface, assisting in uniform pH distribution within the cheese body, and is within the range of 4.5 to 4.7. This process helps in preventing surface layer separation and increasing quality in textural and sensory quality [3].

The functional benefits and extensive use of plastic parchment paper is concerning. Due to being non-biodegradable, being derived from non-renewable petroleum, and needing to be incinerated to be disposed, poses a large risk in conteiminating the environment. Also, polypropylene is prone to no antimicrobial properties and is susceptible to oxidative and microbal degradation [3, 4]. With this in mind, the interest in packaging having biodegrable, sustainable, and active packaging materials has increased.

In the past decades, edible and biodegradable coatings and films applied to be a more sustainable option, compared to conventional packaging materials. Natural polymers, such as proteins, polysaccharides, lipids, or their combinations, form the basis of these films and they're of chemical engineering interest as a result of being biocompatible, cheap, and easily modifiable [2, 5]. Gelatin, starch, gums, fibers, and non-starch polysaccharides are some of the ingredients used to create these films [5].

Biodegradable films do more than act as physical barriers to the passage of moisture, oxygen, and carbon dioxide; they also prevent the migration of lipids and improve the perception of the food they enclose, all while preserving volatile aroma compounds. Because the growth of microorganisms on food surfaces causes food spoilage, the incorporation of antimicrobial or bioactive substances in films can improve the safety of the food and prolong its shelf life [5]. In particular, temporal moisture in a packaged food product is a critical factor to avoid, as it creates an conducive environment for undesirable chemical and enzymatic reactions to occur and compromise the stability of the product [3, 6].

The use of biodegradable films made from agricultural by-products aligns with circular economy and sustainable development principles. Citrus fruits like oranges, mandarins, lemons, and grapefruits are among the most cultivated fruits in the world, and in terms of global citrus production, Iran is

certainly in the top ranks, producing over 3 million tons annually. It is reported that almost 50% of the citrus produced is thrown away, and the majority of it is the peel [4, 6]. In addition to the aforementioned substances in the citrus peel, pectin, dietary fiber, essential oils and bioactive compounds, that makes it suitable for the production of valuable components. Prior research has shown that the peels of oranges and sweet limes do have some prebiotic qualities due to the fiber and pectin [5, 6].

The current study aimed to extend the potential of citrus waste for the production of edible films and the documented role of probiotics in the enhancement of food quality to construct and evaluate the utility of a citrus waste probiotic film as a sustainable; Alternative to parchment paper for the preservation of feta cheese. In order to determine the potential for industrial use of this innovative packaging system, a study was conducted on the microbiological, physicochemical, and sensory characteristics of the feta cheese films and the texture of the cheese during refrigeration.

2. Materials and methods

2.1. Preparation and Activation of Probiotics (for Film Inoculation)

Starting from the Iranian Research Organization for Science and Technology, the lyophilized probiotic strains *Lactobacillus acidophilus* (PTCC 1643), *Lactobacillus plantarum* (PTCC 1896), and *Bifidobacterium bifidum* (PTCC 1644) were acquired. The preparation and activation of the probiotics were carried out as per the explained procedures of Mozaffar et al. (2.020) [7].

2.2. Preparation of Films

Waste materials were sourced from local fruit markets, and sweet lime and orange peels were obtained. The peels were washed, dried, and ground into a powder. The film forming solution was prepared by dispersing 8 gr of the mixed sweet lime and orange peel powder in 100 mL of distilled water. This was followed by heating the mixture to 85 °C for 45 minutes while continuously stirring with a magnetic stirrer. Glycerol was added as a plasticizer at a ratio of 6 mL per 100 mL of the solution. Sunflower seed oil (1% w/w) and Tween 80 were added in the roles of hydrophobic agent and emulsifier, respectively [5]. Bacterial suspensions were prepared with each of the probiotic strains (*L. acidophilus*, *L. plantarum*, and *B. bifidum*) at an initial concentration of 10^9 cfu/mL for incorporation of probiotics. The resulting solutions were poured onto glass Petri dishes and dried in a tray dryer at 50 °C. The films were removed from the plates and immersed in a 2% (v/v) calcium chloride (Sigma-Aldrich, USA) solution for 10 min to improve structural stability. The films were stored under refrigeration until use [7].

2.3. Production of Feta Cheese and Preparation of Treatments

Feta cheese was produced using fresh whole cow's milk pasteurized (at 72 C for 15 s). Prior to cheese making, the milk temperature was adjusted to 35 °C, and 5 L portions were transferred into sterile cheese making containers. A starter culture (Dalton Biotecnologie, Italy) was added at 2% (v/v), followed after 30 min by the addition of calcium chloride at 0.02% (w/v). Rennet (Dalton Biotecnologie, Italy) was then added at 0.001 gr (w/v) and gently stirred for 5 min. The coagulum was cut into 1 cm^3 cubes to enhance rennet efficiency, and the temperature was kept at 35 °C during coagulation. The formed curd was pressed under a 10 kg load for 6 h to facilitate whey drainage. The curd was then cut into uniform 50 gr portions and placed into sterile, lidded containers. A piece of the prepared film was placed on the top of each cheese sample (making sure the cheese was the same thickness for all treatments). The lids were closed, and the samples were kept at 4 °C for 9 weeks (63 days) for further analysis [1].

2.4. Viability of *L. acidophilus, B. bifidum,* and *L. plantarum* in Films

To evaluate the probiotics in the films, 1 gram of each film sample was combined with 9 mL of 0.1% peptone water (Merck, Germany) and vortexed for 10 min. For the 9 week storage period, the

enumeration of Lactobacillus sp and *B. bifidum* was done using MRS (de Man, Rogosa and Sharpe) agar (Merck, Germany) and MRS agar with 0.05% cysteine, respectively. Microaerophilic and anaerobic conditions were used for *L. acidophilus* and *B. bifidum* [7].

2.5. Release of Probiotics from the Film into Feta Cheese

The release of probiotic bacteria from the film into feta cheese was assessed over the storage time. On the chosen sampling days, 10 gr of cheese were aseptically placed into sterile stomacher bags and mixed with 90 mL of 0.1% (w/v) peptone water using a vortex for 10 min. To enumerate the viable Lactobacillus spp. and *B. bifidum* counts, surface plating was performed on MRS agar and MRS agar with 0.05% (w/v) L-cysteine (w/v) respectively. These plates were incubated under specific microaerophilic and anaerobic conditions for the enumeration of Lactobacillus spp. and *B. bifidum*, respectively [7].

2.6. Determination of the Antimicrobial Activity of the Films

The antimicrobial activities of the films were assessed against the following test organisms: *Salmonella Typhimurium* (ATCC 14028), *Listeria monocytogenes* (ATCC 19117), *Staphylococcus aureus* (ATCC 65218), *Escherichia coli* (ATCC 25218), and *Aspergillus flavus*. The microorganisms lyophilized cultures were acquired from the food hygiene lab of the veterinary medicine faculty at the University of Tehran. The cultures were then activated and enriched using Brain Heart Infusion (BHI) broth (Merck, Germany) and subcultured three times to obtain a sufficient viable count. The cultures were standardized to an optical density of 0.1 at 600 nm using a spectrophotometer. Subsequently, the cultures were spread on Mueller Hinton agar (Merck, Germany) plates. The disk diffusion assay was used to test the antimicrobial activities of the films. Disks of the films with a diameter of 10 mm were placed in the center of the agar plates that had been inoculated. Using the disc diffusion technique, the antimicrobial properties were evaluated. A disc of film (10 mm diameter) was made and positioned in the middle of the inoculated agar plates. After the agar plates were incubated for 24 h at 37 °C, the inhibition indexes were noted and measured and were identified as translucent halos around the film discs [7].

2.7. Microbiological and Chemical Analyses of Cheese

2.7.1. Preparation of Cheese Dilutions

To analyze the microbes, 10 grams of the homogenized cheese was placed inside sterile zip bags, which have 90 mL of trisodium citrate solution (2 gr/100 gr). A stomacher (Wiggens, Germany) was used for 2 minutes to homogenize the mixtures. After this, serial dilutions were performed, and the plates were incubated at 37 °C for 72 h. The antimicrobial properties of the films were evaluated at various time periods by counting total coliforms, S. aureus, and molds. During the entire study, samples were stored at 4 °C [1].

2.7.2. Enumeration of *Staphylococcus aureus*

Bairdparker agar (Merck, Germany) was used for Counting as per the Iranian National Standard No. 1924 (Institute of Standards and Industrial Research of Iran). The plates were incubated for 48 h at 37 °C. To confirm the identity of the colonies, coagulase testing was performed.

2.7.3. Enumeration of Total Coliforms

Mean coliform counts were determined using Violet Red Bile Agar (VRBA) (Merck, Germany). Plates were incubated at 37 °C for 24 h [6].

2.7.4. Enumeration of Molds

Mold counts were determined by culturing on PCA agar (Merck, Germany) containing chloramphenicol. Plates were incubated at 25 °C for 24 h [6, 8].

2.7.5. pH and Titratable Acidity

A 10 gr cheese sample was put into a beaker and pH was measured with a calibrated pH meter (Jenway, England) (using buffers solution). For titratable acidity, 10 gr of sample was blended with 40 mL of distilled water at 40 °C, and the mixture was transferred to a 100 mL volumetric flask. Then, 25 mL of the solution was titrated with 0.1 N NaOH (Merck, Germany) (phenolphthalein was added). Titratable acidity was expressed as % lactic acid using the formula [8]:

$$\text{Titratable Acidity (Dornic)} = 4 \times \text{NaOH (mL)} \times 10$$

2.8. Physical and Mechanical Properties of Films

2.8.1. Film Thickness

Different film thickness was measured at 10 random locations using a digital micrometer with 0.0001 mm precision [5, 9].

2.8.2. Moisture Content of Films

After obtaining moisture equilibrium, all films were weighed and the pre weighed capsules. Capsules were dried in an oven at 110 °C until constant weight, then cooled in a desiccator and reweighed [5].

2.8.3. Film Transparency and Opacity Measurements

Film samples were cut into squares and placed inside a spectrophotometer cell. Absorbance spectra were recorded (200–800 nm) [9].

$$\text{Transparency} = \text{Absorbance measured at 600 nm/ Thickness (mm)}$$

2.8.4. Water Vapor Permeability

In accordance with ASTM Method E96, Water vapor transmission rate (WVTR) was obtained [9]. The cells were filled with water and, after being sealed with paraffin at the edges, were placed in a desiccator that contained silica gel at 25 °C and 100% relative humidity. The weighted change of the cell over time was measured with a digital balance (precision of 0.0001 gr). The slope of the curve of weight change in relation to time served to calculate WVTR and was divided by the cell area (0.00287 m^2) [9].

2.8.5. Tensile Strength

As per ASTM D882-91, tensile tests were conducted using a Testometric machine (M350-10CT, UK). strips of film (10 × 1 cm^2) were pulled at a jaw separation distance of 50 mm and a crosshead speed of 50 mm/min [9].

2.9. Sensory Evaluation

Using a 5 point hedonic scale, the sensory acceptability of samples of cheese was assessed. Each cheese block (100 gr) was divided into 7 pieces and each piece was coded randomly. The pieces were served to a trained panel of 9 evaluators. Color, appearance, aroma, and taste were the assessed attributes [4].

2.10. Statistical analysis

Statistical analysis has been carried out using the SPSS version 25 software. Initially, the data was subjected to the Kolmogorav-Smirnov test to check its normality, and subsequently the Leven test was employed to check the homogeneity of the data's variance. To assess the means of the number of bacteria across the different time periods of the study across the various groups, repeated measures ANOVA was used. In the case of sensory evaluation, which is a qualitative variable, Friedman test was applied.

3. Results

3.1. Viability of *L. acidophilus*, *B. bifidum* and *L. plantarum* in Films

Different film formulations were monitored throughout the storage period to check the viability of the probiotic bacteria *L. acidophilus*, *B. bifidum*, and *L. plantarum*. In Figure 1, a gradual decline in viability was observed, however the reduction varied among the different film formulations. On day 0, the recorded initial counts of *L. acidophilus*, *B. bifidum*, and *L. plantarum* were 8.90, 8.87, and 8.88 Log cfu/g, showing no significant difference among the different probiotic strains (P>0.05). *B. bifidum* showed a significantly lower viability compared to the other two strains by day 14 (P<0.05).

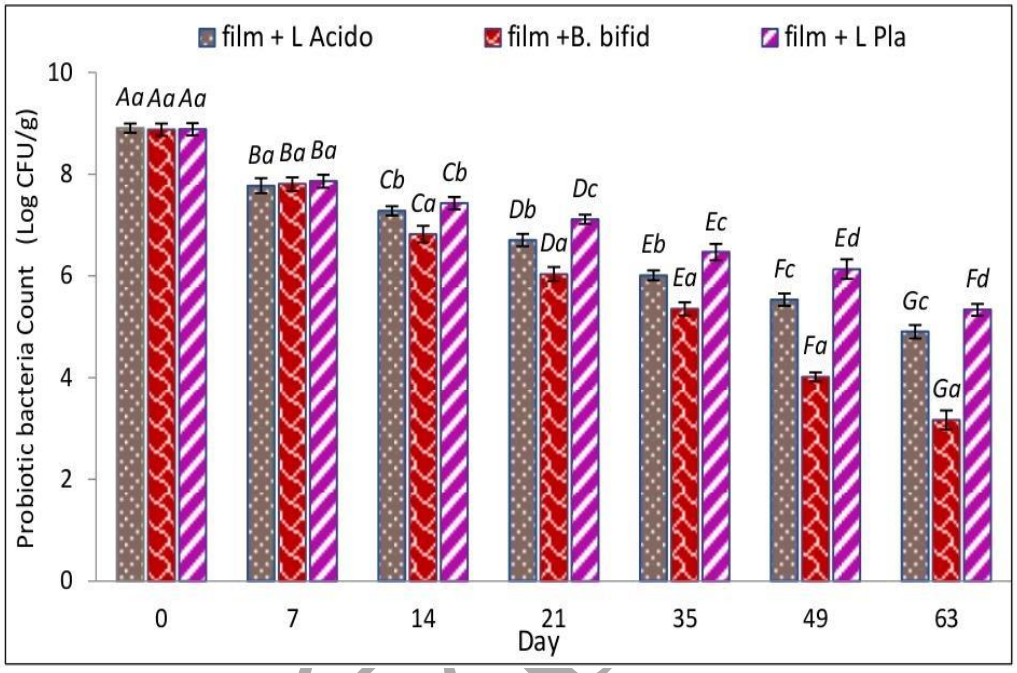

Figure 1. Results of the viability of probiotic bacteria in various films during storage (Log cfu/g).

After 63 days of storage, there was a decline in the viable counts of *L. acidophilus*, *B. bifidum*, and *L. plantarum*, which decreased to 4.90, 3.16, and 5.33 Log cfu/g respectively, indicating a gradual loss of viability over time. Significant differences in final counts among the three probiotics were observed, with *L. plantarum* showing the highest viability and *B. bifidum* the lowest (P<0.05).

3.2. Release of Probiotics from the Film into Feta Cheese

The transfer of probiotics from the film to feta cheese is depicted in Figure 2. Probiotics were not released on days 0 and 7 of storage. However, on day 14, *L. acidophilus* and *L. plantarum* were released with counts of 1.18 and 1.32 Log cfu/g, respectively, having no significant difference (P>0.05). Starting on day 35, there was release of *B. bifidum* into the feta cheese which was 1.19 Log cfu/g. On day 63, the viable counts of *B. bifidum*, *L. acidophilus* and *L. plantarum* that were released into the feta cheese were 1.56, 2.23 and 3.95 Log cfu/g respectively. Among the three probiotic strains, final population numbers showed statistically significant differences. *L. plantarum* reached the highest population levels, while *B. bifidum* reached the lowest (P<0.05).

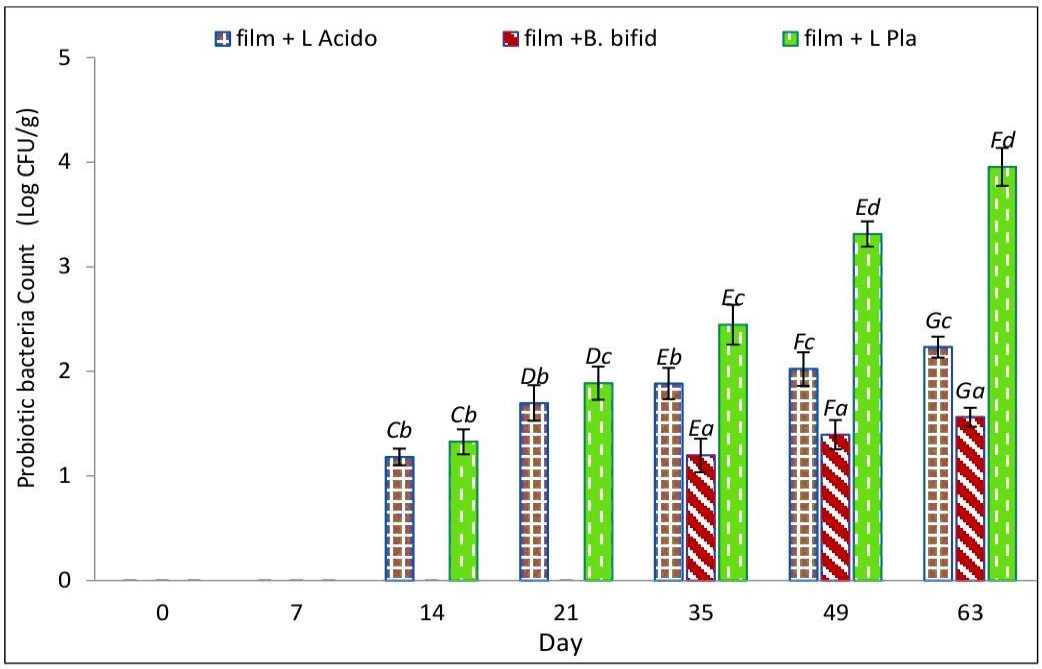

Figure 2. Results of the release of probiotics from the film into feta cheese (Log cfu/g).

3.3. Antimicrobial Activity of the Films

The antimicrobial activities of the different films against pathogenic microorganisms are shown in Figure 3. The films showed disparate levels of antimicrobial activity against *S. Typhimurium*, *L. monocytogenes*, *E. coli*, *S. aureus*, and *Aspergillus flavus* ($P<0.05$). In the case of *L. monocytogenes*, the highest zone of inhibition (19.07 mm) was recorded for the film containing *L. acidophilus*, which was significantly greater than the films containing *L. acidophilus* and *B. bifidum*. The films containing *L. acidophilus* and *B. bifidum* showed zones of inhibition of 8.07 mm and 2.52 mm, respectively, which was significantly different ($P<0.05$). The non-probiotic film showed no anti listerial activity.

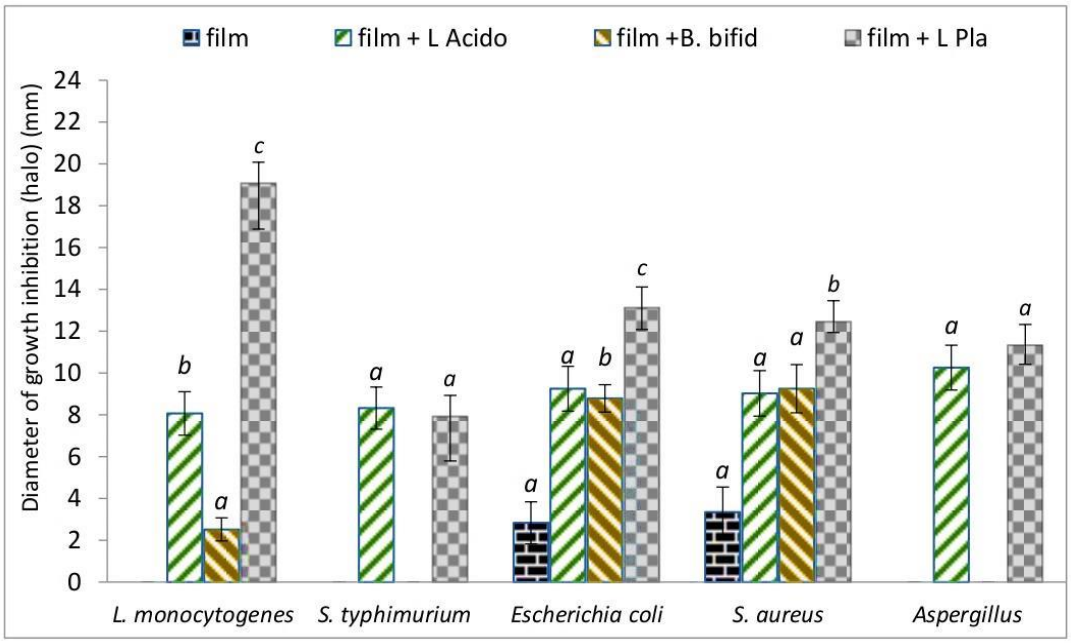

Figure 3. Results of the evaluation of antimicrobial activity and growth inhibition (halo) of films prepared from citrus waste against selected pathogenic microorganisms (mean ± SD).

For *Salmonella Typhimurium*, the films containing *B. bifidum* and the non-probiotic film showed no activity, while the films containing *L. acidophilus* and *L. plantarum* had comparable inhibition zones (P>0.05). In general, the non-probiotic film showed antimicrobial activity only against *E. coli* (2.83 mm) and *S. aureus* (3.35 mm) at levels significantly lower than the films containing probiotics (P<0.05). The film with *L. plantarum* showed the greatest inhibition zones against *E. coli* and *S. aureus* (P<0.05) compared to the films with *L. acidophilus* and *B. bifidum* that showed comparable activity (P>0.05). For *Aspergillus flavus*, only the films with *L. acidophilus* and *L. plantarum* had antifungal activity with mean inhibition zone diameters of 10.25 mm and 11.32 mm, respectively and not significantly different (P>0.05) of each other.

3.4. *Staphylococcus aureus* Enumeration

Figure 4 shows the effect of different citrus waste based films compared to parchment paper on the counts of coagulase positive *S. aureus* in Feta cheese. The data showed that only the films containing the probiotics significantly impacted the reduction of *S. aureus* viability after 63 days of storage compared to the plain film (probiotic free) and parchment paper (P<0.05). Overall, the treated films shown a general upward trend, however, the greatest quantity of *S. aureus* was noted in the non-probiotic film and parchment paper groups. For the course of the study *S. aureus* levels in the parchment paper group and non-probiotic film group ranged from 2.63 Log cfu/g and 2.69 Log cfu/g at the beginning, to 6.32 Log cfu/g and 6.21 Log cfu/g, respectively, at the end. The counts obtained in the non-probiotic film and parchment paper groups were significantly different than those obtained in the probiotic film groups, but the counts obtained in the two (non-probiotic film and parchment paper groups) were not significantly different (P>0.05). The probiotic films showed varying results where *L. plantarum* film showed the best results in controlling *S. aureus*. The *S. aureus* counts increased from 2.57 Log cfu/g at the start to 4.22 Log cfu/g by the end of the study and was significantly different than the other groups (P<0.05). The films containing *L. acidophilus* and *B. bifidum* exhibited similar results with no significant difference between the two (P>0.05).

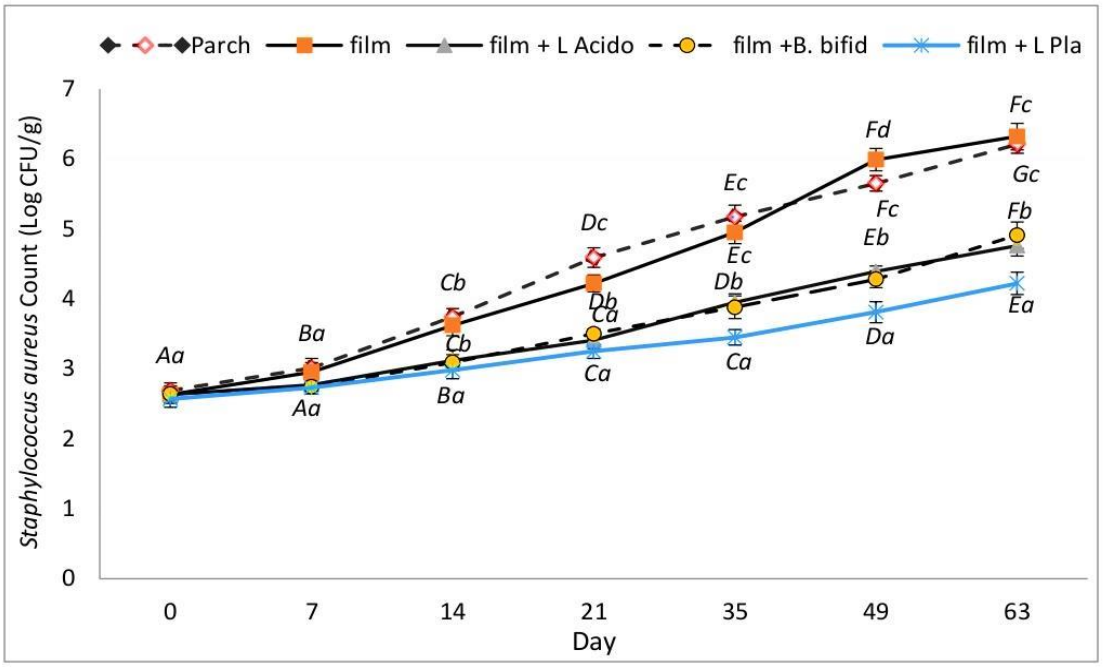

Figure 4. Results of the effect of different films on changes in *Staphylococcus aureus* counts in feta cheese during storage (Log cfu/g).

3.5. Total Coliforms Enumeration in Feta Cheese

In the Figure 5, different citrus waste based films were compared to parchment paper on total coliform counts in Feta cheese (sliced). The results obtained have shown an increase in coliform populations across all treatments during storage. There was no significant difference found between the three groups: the films containing *L. acidophilus* and *B. bifidum*, the parchment paper, and the no-probiotic films (P>0.05) regarding the coliform counts. Coliform counts for the films containing *L. acidophilus* and *B. bifidum* increased during the storage period from 2.29 Log cfu/g and 2.20 Log cfu/g to 3.02 Log cfu/g and 3.13 Log cfu/g (P>0.05). The films containing *L. plantarum*, however, were able to achieve the best results in controlling coliform counts as there was a significant difference in coliform growth when compared to all the other groups (P<0.05). The coliform counts increased only from 2.22 Log cfu/g to 2.75 Log cfu/g demonstrating that there was only a slight increase in coliforms.

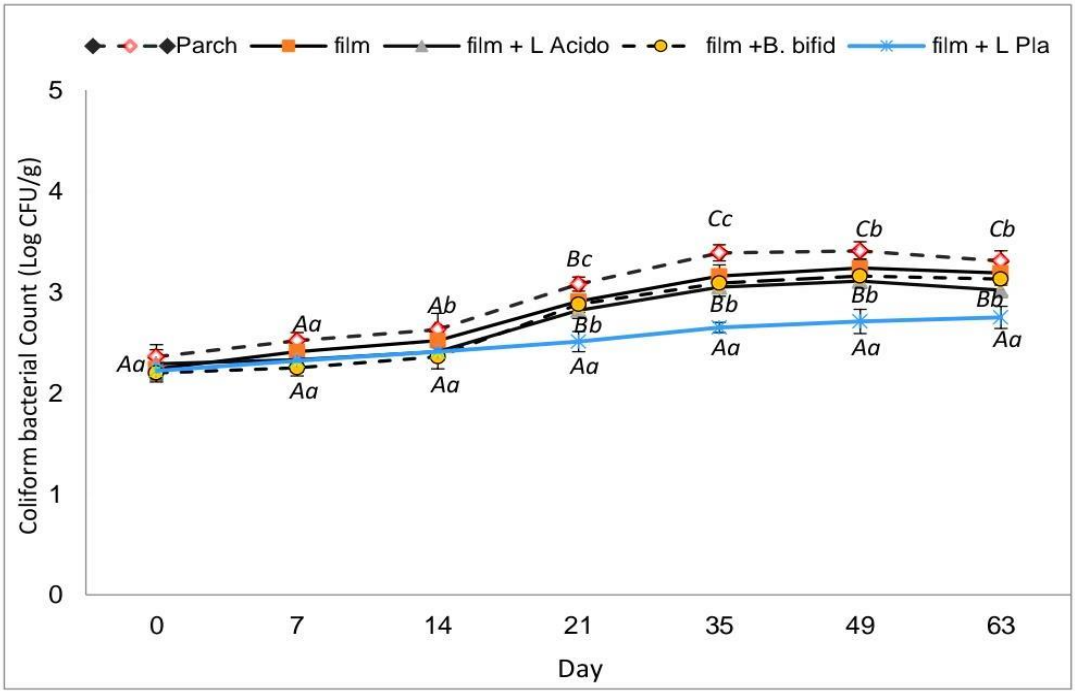

Figure 5. Results of the effect of different films on changes in total coliform populations in feta cheese during storage (Log cfu/g).

3.6. Mold Growth Changes in Feta Cheese

The growth of mold was studied over a period of 63 days in Feta cheese covered with the different films, which include the non-probiotic films, the films containing the probiotics *L. Acidophilus* and *B. bifidum*, and *L. plantarum*, and parchment paper (Figure 6). In all storage treatments, the population of molds increased over the storage period. The highest increase in mold growth was found in the non-probiotic film group, from 1.18 Log cfu/g to 5.96 Log cfu/g. Films containing *L. acidophilus* and *B. bifidum* markedly decreased mold growth in contrast to the non-probiotic film (P<0.05). Parchment paper was better than films containing *L. acidophilus*, *B. bifidum*, and *L. plantarum* in controlling mold. However, the best treatment was the film containing *L. plantarum*, where mold counts increased only from 1.21 Log cfu/g at day 0 to 3.56 Log cfu/g at day 63 (P<0.05). This indicates that it had the least mold.

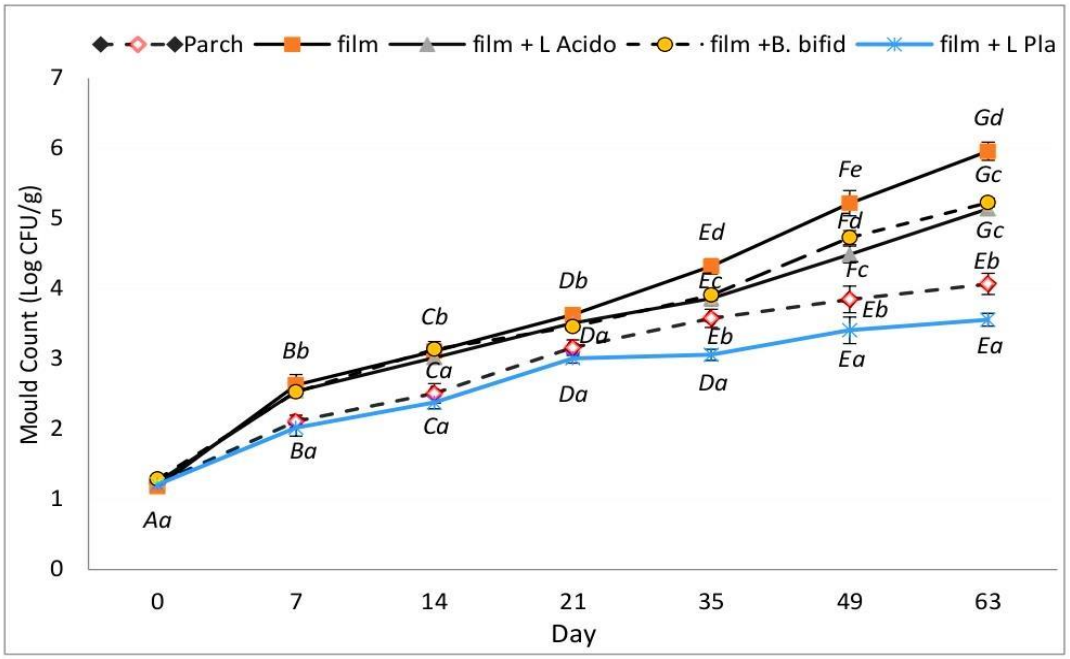

Figure 6. Results of the effect of different films on mold counts in feta cheese during storage (Log cfu/g).

3.7. pH and Acidity

The pH results are given in Table 1. The results show that over 63 days, pH decreased for Feta cheese samples covered with probiotic films. The lowest pH values were observed in cheeses coated with *L. acidophilus* and *L. plantarum* films, decreasing from 4.63 and 4.60 on day 0 to 4.44 and 4.43 on day 63, showing comparable results (P>0.05). Cheeses with *B. bifidum* films had only minor pH changes. Cheeses wrapped with parchment paper and the non-probiotic film had pH increases, rising from 4.62 and 4.65 on day 0 to 4.89 and 4.86 on the last day, respectively, showing comparable trends (P>0.05).

Table 2 outlines the changes in acidity. All samples exhibited a gradual increase in acidity during the storage period. Parchment paper and non-probiotic film groups showed no significant changes (P>0.05). In contrast, probiotic film cheeses exhibited the greatest acidity after storage (P<0.05). The acidity for cheeses wrapped in films of *L. acidophilus* and *L. plantarum* increased from 0.74 and 0.72 on day 0 to 1.80 and 1.88 on the last day, respectively, which showed comparable results (P>0.05).

Table 1. Average pH changes in different treatments (mean ± SD).

Treatment / Day	Parch	film	film + L. Acido	film +B. bifid	film + L. Plan
0	4.62 ±0.05^{Aa}	4.65±0.02^{Aa}	4.63±0.03^{Aa}	4.61±0.02^{Aa}	4.60±0.03^{Aa}
7	4.66±0.04^{Aa}	4.67±0.01^{Aa}	4.65±0.01^{Aa}	4.64±0.02^{Aa}	4.63±0.02^{Aa}
14	4.67±0.01^{Aa}	4.70±0.02^{Ba}	4.68±0.03^{Aa}	4.66±0.02^{Aa}	4.67±0.01^{Aa}
21	4.71±0.03^{Ab}	4.72±0.01^{Bb}	4.61±0.01^{Aa}	4.65±0.00^{Aa}	4.63±0.02^{Aa}
35	4.77±0.02^{Bb}	4.80±0.03^{Cb}	4.56±0.05^{Aa}	4.63±0.02^{Aa}	4.58±0.01^{Aa}
49	4.85±0.03^{Cd}	4.83±0.01^{Cd}	4.50±0.01^{Ab}	4.61±0.00^{Ac}	4.47±0.00^{Ba}
63	4.89±0.04^{Cc}	4.86±0.01^{Dc}	4.44±0.03^{Ba}	4.55±0.02^{Bb}	4.43±0.01^{Ca}

Different lowercase letters indicate a significant difference between different treatments on the same day (P<0.05), and different uppercase letters indicate a significant difference for the same treatment across different days (P<0.05).

Table 2. Average pH changes in different treatments (mean ± SD).

Treatment / Day	Parch	film	film + L. Acido	film +B. bifid	film + L. Plan
0	0.73 ±0.02^{Aa}	0.77 ±0.01^{Aa}	0.74 ±0.04^{Aa}	0.75 ±0.02^{Aa}	0.72 ±0.03^{Aa}
7	0.75 ±0.05^{Aa}	0.80 ±0.03^{Aa}	0.81 ±0.03^{Aa}	0.82 ±0.02^{Ba}	0.83 ±0.04^{Ba}
14	0.79 ±0.03^{Aa}	0.82 ±0.02^{Aa}	0.94 ±0.04^{Bb}	0.91 ±0.01^{Cb}	0.98 ±0.01^{Cb}
21	0.88 ±0.00^{Ba}	0.88 ±0.01^{Ba}	1.13 ±0.02^{Cb}	1.08 ±0.03^{Db}	1.16 ±0.06^{Db}
35	0.91 ±0.01^{Ca}	0.92 ±0.03^{Ba}	1.45 ±0.02^{Dc}	1.35 ±0.02^{Eb}	1.53 ±0.05^{Ed}
49	0.95 ±0.04^{Ca}	0.97 ±0.02^{Ba}	1.61 ±0.01^{Ec}	1.50 ±0.02^{Fb}	1.63 ±0.02^{Fc}
63	1.03 ±0.01^{Da}	1.01 ±0.00^{Ca}	1.80 ±0.01^{Fc}	1.65 ±0.02^{Gb}	1.88 ±0.07^{Gc}

Different lowercase letters indicate a significant difference between different treatments on the same day (P<0.05), and different uppercase letters indicate a significant difference for the same treatment across different days (P<0.05).

3.8. Physical Properties of Films

The results of the analysis for each type of film's physical properties are in Figures 7 and 8. The probiotic addition to the films affected film clarity (opacity) and the rate of water vapor transmission when compared to non probiotic film (P<0.05). The combination of *L. acidophilus*, *B. bifidum*, and *L. plantarum* resulted in less water vapor permeability and increased film clarity. Within the probiotic films, however, no significant difference was identified (P>0.05). There were no significant differences in tensile strength, moisture content, and thickness of the films (P>0.05).

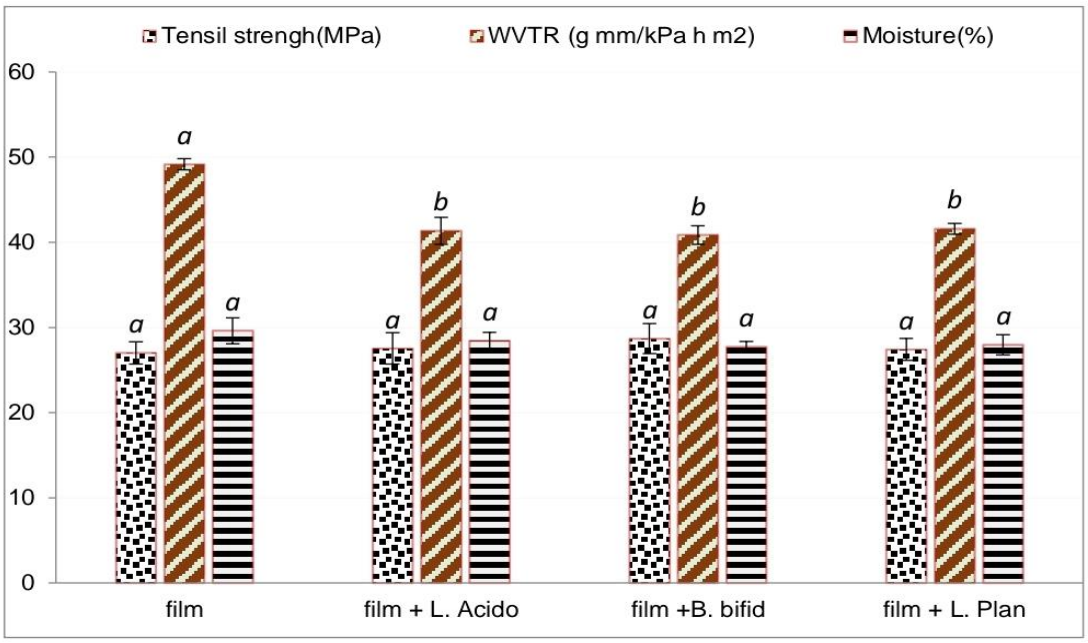

Figure 7. Results of the evaluation of tensile strength, water vapor permeability, moisture content, and swelling index in films prepared from citrus waste (mean ± SD).

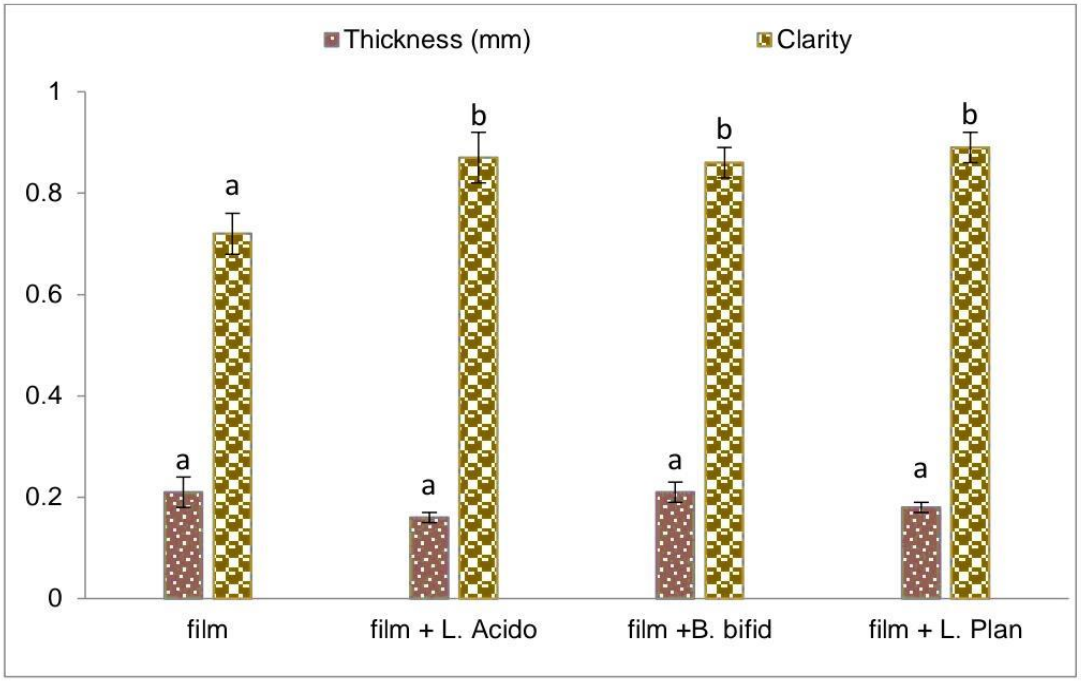

Figure 8. Results of the evaluation of thickness and clarity in films prepared from citrus waste (mean ± SD).

3.9. Sensory Evaluation

The samples of feta cheese were evaluated for sensory attributes (aroma, texture, taste, color, and overall acceptance). The feta cheese samples were coated with different types of materials (parchment paper, non-probiotic and probiotic films containing *L. acidophilus*, *B. bifidum*, and *L. plantarum*). The results showed that *L. acidophilus* and *L. plantarum* had the highest scores (5 each), while the samples with only parchment paper had the lowest scores (3). In texture, *L. plantarum* films had the highest score (5) while the samples that had only parchment paper had the lowest score (3.5) ($P<0.05$). For color, the samples that had parchment paper had the highest score while both probiotic and non-probiotic films had lower scores that were not significantly different from each other ($P>0.05$). Overall, there were significant differences ($P<0.05$) and *L. plantarum* films were rated the highest while parchment paper was rated the lowest.

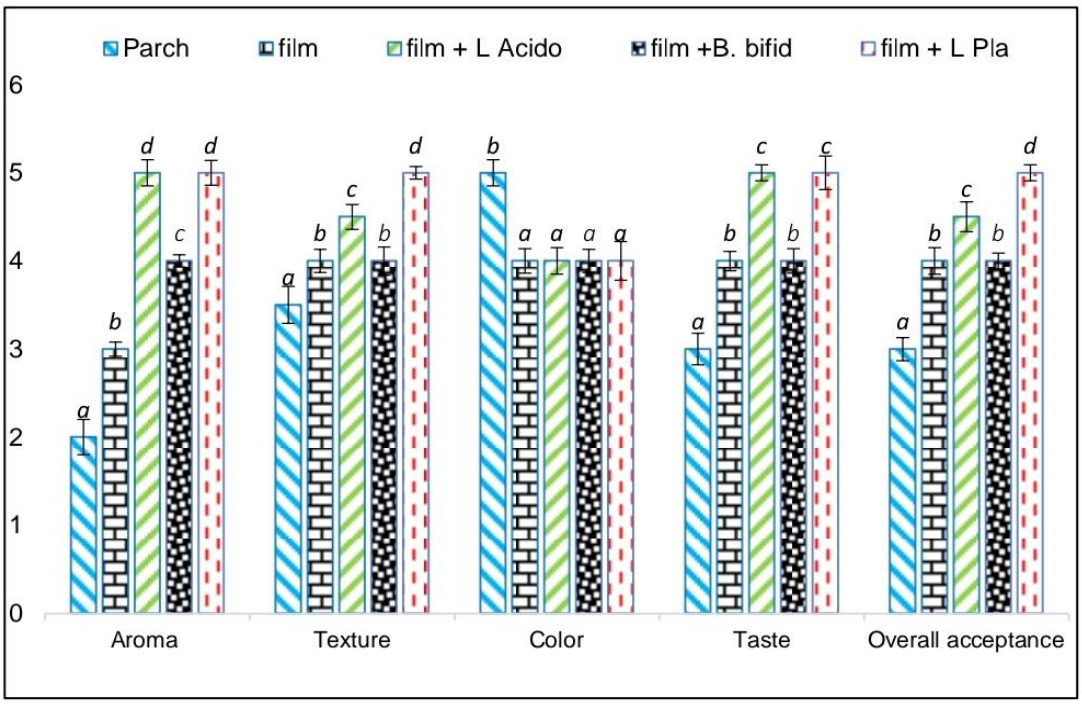

Figure 9. Sensory evaluation results in different treatments during storage.

4. Discussion

4.1. Viability of Probiotics in the Films

The results of the present study show that *L. acidophilus*, *B. bifidum*, and *L. plantarum* proved to be viable in the citrus waste-based films, however, they did not remain viable for the entire 9 week period. Documented reductions in the viability of probiotics encased in edible films have been attributed by many researchers to osmotic stress, decreased water activity, reduced nutrient diffusion, limited oxygen, and the films trapping certain acidic by-products [8].

The comparability of initial counts of remaining probiotics on day 0 shows uniform inoculation and favorable initial conditions for the creation of the films for the three strains. This shows the citrus waste-based film matrix offered an initial conducive environment for the probiotics that supports previous findings of polysaccharide and fiber-based films [9]. Over time, with the passage of time, and most noticeable after day 14, considerable strains of the films were recorded, of which the increased concentration of organic acid and moisture, and the reduction of gas exchange, were probably the most major contributors [10].

Among the strains, the lowest stability of all was that of the *B. bifidum* strain. The major reason for *B. bifidum* strain losing viability first can be pointed to the strain being more anaerobic compared to the rest of the strains, thus making it more sensitive to the exposure of oxygen [11]. Bifidobacteria generally show weaker resistance to changes of pH and water activity than lactobacilli, especially in edible film systems that are semi-dry [12]. *L. plantarum* showed the greatest viability at the end of the storage period. This greater viability could be attributed to its high acid tolerance, flexible fermentation of plant and fibrous materials, and the ability to withstand diverse environmental challenges [13]. *L. plantarum* could metabolize some of the complex carbohydrates and fibers, including pectin, that are found in some citrus wastes and this helped sustain the viability of *L. plantarum* in the film matrix, which is also noted in other works involving synbiotics [14].

L. acidophilus had medium viability. While storage conditions resulted in significant loss of viability, *L. acidophilus* had greater viability than *B. bifidum*. This is consistent with previous findings

that showed greater survivability in the environment of lactobacilli that are incorporated in edible films and biodegradable coatings [15].

From an application point of view, these findings demonstrate the importance of the right choice of probiotic strains when developing functional active films. The high end-storage counts of *L. plantarum* also justify its inclusion in the functional food product as a probiotic active constituent, as the count surpasses the functional threshold value [14, 15].

4.2. Release of Probiotics from the Film into Feta Cheese

The probiotic release profile from the citrus waste-based films into feta cheese in this study shows that there is a gradual migration of viable cells from the film matrix to the cheese surface throughout the duration of the storage. The delays in the release of proteolytic from the film matrix to the cheese surface observed at the beginning (0 days and 7 days) is likely due to a strong retention of the probiotic cells to the polymeric matrix of the film, which impeded diffusion in the early stages. A similar phenomenon of delayed release has been noted in studies on edible films encapsulating probiotics, where the structure of the film serves as a protective barrier that provides controlled microbial migration to the surrounding matrix over time [8].

Observations of the release of probiotic strains *L. acidophilus* and *L. plantarum* on the 14th day of storage, and the absence of statistically significant difference of the amount of the two strains in the feta cheese, suggests that these strains adapted and migrated into the cheese matrix at a similar rate during the early period of storage. However, the release of *B. bifidum* was observed on the 35th day of storage, which can possibly be attributed to the higher sensitivity of this strain to the oxygen, salt, and acidity present in the feta cheese [11]. The microclimate surrounding *B. bifidum* in the film may have protected this strain from those conditions, which may have delayed the migration of *B. bifidum* into the cheese matrix.

At the end of storage, *L. plantarum* had appreciably more viable counts than the rest of the strains. This agrees with earlier studies that recognize *L. plantarum* dominance in dairy systems due to the low pH and high salt levels [8, 11]. On the other hand, the lower release and survival of *B. bifidum* could represent its lack of ability to withstand such stress conditions, even with the encapsulation within the film.

4.3. Antimicrobial Activity of Probiotic Films

This study demonstrates that the addition of probiotics into the composition of biodegradable films results in the development of antimicrobial properties, which are active, and also, which are caused by the certain strain. This phenomenon is consistent with the notion of active films, wherein the living cells present in the film can contribute to the suppression of the pathogenic microorganisms by producing antimicrobial compounds and altering the conditions of the surface of the food. Other researchers have reported that the presence of lactic acid bacteria in films increases the effectiveness of the films in preventing foodborne pathogens, as opposed to films without bacteria [8, 9].

The physiological traits of probiotic strains may explain why they respond differently to each of the pathogens. *L. plantarum* is known to be one of the most effective strains with the ability to produce a wide variety of organic compounds and antimicrobial peptides. This helps the strain. with inhibitory activities against most Gram-positive and some Gram-negative pathogens [13, 16]. In comparison, bifidobacteria are more influenced by the surrounding environmental conditions of the films, which may explain the lower efficacy of bifidobacteria, as they exhibit a narrower antimicrobial spectrum [11].

The various actions of the films against the different pathogens can also be explained by microbiological principles. Naturally, due to the more complicated cell walls with outer membranes that are rich in lipopolysaccharides, Gram-negative bacteria are more difficult to kill. This usually means that such groups require probiotics with a higher metabolic activity or a more intense microbial

production, a characteristic that is more evident in the case of lactobacilli than in bifidobacteria [14, 17].

In films that lack probiotics, some antimicrobial activity could be due to the waste matrix's bioactive compounds. Although polyphenols and other phenolic compounds, as well as remaining essential oils, have antimicrobial activity, the strength of the effect of each of these compounds is typically less than that of probiotic and fiber composite systems. Probiotics and polyphenols may work synergistically to enhance the antimicrobial activity of the films [9, 17].

As for antifungal, the films' ability to control lactobacilli can be attributed to the production of certain antifungal metabolites. Lactobacilli secreted phenyl lactic acid and other low molecular weight metabolites that inhibit mycotoxin-producing fungi, and bifidobacteria, in contrast, have little ability to synthesize these inhibitory compounds [18].

4.4. Changes in *Staphylococcus aureus* Population in Feta Cheese

The type of coating used is critical in controlling the population of coagulase positive *S. aureus* in feta cheese. The *S. aureus* counts' increasing trend was seen for all the treatments, however, the presence of probiotics in the film was shown to have an impact on the extent of this increase. *S. aureus* growth is supported by intrinsic properties of feta cheese. It is high pH, has suitable water activity and nutrients [19].

The poor performance of films and parchment sheets without probiotics demonstrates that passive packaging only protects the cheese, and does not change the biotic surface microenvironment. They can retain moisture and pH, but without intrinsic antimicrobial activity, they will not protect the cheese from pathogen proliferation. Using only inactive packaging does not stop the growth of *S. aureus* in soft cheeses [19, 20].

In contrast, films with probiotics showed functional activity by significantly reducing *S. aureus* growth. Less microbial activity may have been the result of localized cheese surface pH, organic acid production, nutrient and space production, and antimicrobial secretions of the the cheese [19, 20]. These attributes are important in environments like cheese surface, where fungi and bacteria multiply.

The enterotoxins produced by *Staphylococcus aureus* are one of the many reasons why the complete reduction of Staph bacteria from food products is not a valid criterion from a food safety standpoint. The enterotoxins are a serious health concern and are not removed by bacterial reduction. Therefore, the overall concern for the food product can be managed by simply slowing the growth of the pathogen during storage [19, 20].

4.5. Changes in Coliform Populations in Feta Cheese

The growth of coliforms, as macroscopic indicators of hygiene, is correlated with the presence of Gram negative bacteria, and during storage, it can indicate secondary contamination and varying processing conditions. According to the findings of this research, although the overall population of coliforms in all treatments was increasing, the type of film used was a major factor in the total amount of this increasing population. The higher water activity and softer texture of the cheese contributes to the more gradual growth of the coliforms, even in the presence of salt [1, 19].

The similar lack of a significant difference among the treatments with parchment paper, the film without probiotics, and the films with *L. acidophilus* and *B. bifidum* may indicate that these systems have a similar inability to inhibit Gram negative indicator bacteria. This may be caused by the inherent resilience of coliforms. gram negative bacteria have less permeable membranes. They have an outer membrane that has lipopolysaccharide structures that make less permeable the cells that other antimicrobials and even organic acids, and that therefore less active many of the lactic acid bacteria [11, 17].

The citrus waste matrix may be responsible for the strengthening of probiotic effects. Synergism is possible with the probiotics for the phenolic compounds, the pectin and the other active compounds of

the citrus peels. While these compounds individually do not have strong coliforms inhibiting activity, in conjunction with the metabolites of *L. plantarum*, they can set unfavorable conditions for the multiplication of these microbes [9]. From the standpoint of safety and quality of the food, the paramount importance of the control of population of coliforms, is that the increase in this indicator is directly related to the deterioration of the hygienic quality of the food and the increase of the risk of the presence of other Gram negative pathogens. In this sense, the simple fact of reducing the growth rate of coliforms during storage represents an important benefit, particularly for traditional and semi-traditional cheeses, such as the case of feta, which are stored for long periods of time in refrigerated conditions [20].

4.6. Changes in growth of Mold in Feta Cheese

The microorganisms that develop in Feta cheese are among the most prominent factors that contribute to the decreasing shelf-life of this product. This is because they are able to develop even in cold storage, as long as the water activity is sufficiently high and the product contains salt. Results showed that there were increases in the growth of all treatments, however, the degree to which growth occurs is different between treatments and is dependent largely on the coating type and inclusion of the probiotics. With no active agents, complete inhibition of the growth of all of the molds is impossible, which is consistent with other reports on brined and soft cheeses [1, 19].

The results also indicate that there were optimal conditions to which the coating without probiotics performed better. This was expected, as the coating was constructed with biodegradable polymers, and the coating by itself, devoid of probiotics, would be incapable of limiting microorganisms on the fungal side. This finding also corroborates that the inactive films are mainly serving as physical barriers, and without a chemical and biological activity within the cheese, the films are just barriers. The molds, when left undisturbed, continue to develop on the cheese and utilize surface moisture and the nutrients that are available [19].

The samples covered with the films containing *L. acidophilus* and *B. bifidum* which show less mold growth demonstrate that the lactic acid bacteria have some potential to inhibit fungi. This is mainly due to the lactic acid bacteria antifungal metabolite production which includes hydrogen peroxide, organic acids, and some other volatiles which limit fungal growth and germination of fungal spores [18]. This effect in these strains was most likely not strong because of the limited antifungal compound producing ability, the environmental conditions on the cheese surface that these bacteria strains are sensitive to, or some combination of the above.

More probiotics films had worse mold growth inhibition than some parchment films. The most probable reason is that the mold growth is anaerobically inhibited by the parchment's ability to limit gas transport, combined with moderate gas permeabilities which would reduce fungal spores to some extent, and the lack of antimicrobial activity. More molds are aerobic than anaerobic. Therefore, gas diffusion limits their growth, even without the presence of antimicrobial agents [1, 19].

Among biodegradable films, the one with *L. plantarum* is the most impressive, which means this strain is the most effective at inhibiting molds. *L. plantarum* is among the strongest antifungal lactic acid bacteria with the ability to biosynthesize antifungal metabolites like phenyl lactic acid, hydroxyphenyl lactic acid, and other low molecular mass metabolites that inhibit spoilage molds [13, 18]. In addition, the optimum compatibility of lactic acid bacteria with the fiber-rich citrus waste matrices may, in these case, help sustain the bacterium’s antifungal activity in the form of stable metabolic activity throughout the storage period.

From a practical perspective, the antifungal activity of *L. plantarum* is important to control spoilage and sustaining the sensory quality of the feta cheese. Some molds grow on food and biosynthesize mycotoxins that are injurious to the health of the consumers. For these reasons, the

antifungal activity of *L. plantarum* that reduces the rate of mold growth during storage, is important to increase the safety and shelf life of feta cheese [17].

4.7. Changes in pH and Acidity of Feta Cheese

The reduction in pH resulting from the coating of samples with probiotic films is caused by the activity of the lactic acid bacteria and the formation of organic acids. The bacteria *L. acidophilus* and *L. plantarum* complete fermentation of lactose and other sugars, resulting in the formation of lactic acid and other acidic byproducts. This leads to a consistent reduction of the cheese's surface pH and bulk pH. This is consistent with earlier studies done on soft and brined cheeses [19, 21].

On the other hand, the rise in pH levels of samples with parchment paper and films that do not contain probiotics can be attributed to the lack of active acid-producing bacteria and the dominance of secondary protein decomposition. In these situations, protein decomposition and the liberation of alkaline materials like amines and ammonia can elevate the pH. This has been documented in studies involving the aging of feta cheese and other cheeses with a higher salt content, where the lack of active microbial life results in a shift of the acid-base balance of the cheese toward an alkaline state [21].

The film with *B. bifidum* also exhibited interesting results. The minor drop in pH in this treatment is probably a consequence of bifidobacteria's sensitivity to the prevailing environmental factors, including the presence of oxygen, salt, and the initial pH of feta cheese. There is some evidence that Bifidobacteria show increased acidifying activities in microaerophilic and certain dairy environments, although their fatty acid metabolizing activities may be more limited on cheese surfaces [21].

Increased acidity in the probiotic containing samples confirms the accumulation of organic acids during storage. More pronounced final acidity in the probiotic treatments also suggests that the microorganisms, in addition to lowering pH, help to create a more consistently stable acidic condition in the cheese. Such conditions would serve to limit the growth of some spoilage and pathogenic organisms, which corresponds to the microbiology of this study [18].

The limited reduction in pH and controlled slight increase in acidity in feta cheese is beneficial from a technical perspective. Increased microbial safety is achieved with a lower pH, without greatly compromising sensory quality. Feta cheese has been shown to offer the best compromise between safety, texture, and taste in the pH range of about 4.4 4.8 [21]. Thus, probiotic films, in particular those with *L. acidophilus* and *L. plantarum*, perform well in these aspects.

4.8. Physical Properties of Probiotic Containing Films

Clarity and water vapor permeability (WVP) of packaging films is critical to their function as protective coatings for food. Variations in these properties and their ranges are important to consider for microbial stability, sensory quality, and consumer acceptance. With respect to biodegradable films containing probiotics, some physical properties were altered while others remained the same, as was the case for several mechanical and structural attributes.

Reduced water vapor permeability in probiotic containing films is likely a result of the physical and chemical interactions of the bacterial cells and the polymer chain structure of the film. Probiotic cells such as *L. acidophilus*, *B. bifidum*, and *L. plantarum* have been demonstrated to function as bio fillers, filling spaces in the polymer and impeding the pathways for water molecule diffusion. Numerous studies have demonstrated the phenomenon where incorporation of a biological material in a polysaccharide or protein composite film, enhanced its microstructure and decreased permeability [22, 23]. A more even distribution of film constituents as well as better microstructural uniformity would explain the improved clarity. The more even the distribution of cells within the polymer matrix, the more the light scattering will be minimized, thus, improving the clarity of the film. This is important as it allows consumers to see the product better, thus, aiding in the marketability of the

product. Active film studies have shown that optical clarity of the films is directly related to the structural homogeneity of the films [22].

Because there were no notable moisture content, film thickness, and tensile strength changes, the addition of probiotics did not alter the mechanical strength of the films. This is important in an industrial context as one of the major obstacles in the development of active films is the incorporation of biological and/or antimicrobial components while still retaining mechanical strength and functionality. In this instance, the probiotics, at the given concentrations, did improve some functional characteristics of the films without compromising the continuous structure of the polymer matrix. Other studies have also shown that this held true for films with various other bioactive components [23]. Packaging of feta cheese now achieves the dual benefits of increased clarity and lower water vapor permeability. Concerning moisture changes, surface spoilage microorganisms are limited in growth because moisture is not transferred, while the product's clarity is improved, thereby maintaining its quality. These benefits are better than regular parchment sheets, which do are paper without any functionality and do not have moisture control [24].

4.9. Sensory Evaluation

The results showed that the presence of probiotics in biodegradable films positively impacted the aroma, flavor, and texture of cheeses, and the color of the cheese was unaffected. The samples with *L. acidophilus* and *L. plantarum* had more aroma and flavor and this is because of the scent molecules of these strains. Lactic acid bacteria produce organic acids, esters, and other compounds that are positive for the cheese to give the cheese a better taste and aroma [25]. The results are the same for the other studies that indicate that probiotics and active bacteria affect the sensory properties of soft and semi hard cheeses.

With regard to texture, the samples with *L. plantarum* received more scores and this is because of the cheese holding moisture and because of the the structure of the cheese matrix due to the formation of acid and the interaction with the protein of the matrix. The probiotics resulting in controlled pH reduction and subsequent acidification leads to the partial coagulation of proteins, preserving the firmness and textural integrity [21].

The effect, however, was less pronounced in parchment wrapped samples, most likely due to the lack of active microbial activity as well as the increased surface moisture accumulation in which the microbial activity could have been.

The overall acceptance of the samples reflected the synergistic effect of the probiotic films, especially those with *L. plantarum*, which attained the highest ratings. This was in line with other findings in the study, where effective microbial control, reduction of pH, appropriate acidification, improved texture, and increased aroma and flavor combined to provide a greater sensory experience with subsequent increased consumer acceptance [18, 25].

5. Conclusion

The findings of the current work show that probiotic active biodegradable films based on citrus waste, preserves feta cheese from spoilage and pathogenic microorganisms, improves the textural and sensory attributes of feta cheese, and possess favorable plastic properties of clarity and low water vapor transmission rate. Among the probiotics used, *L. plantarum* was the most effective and resulted in enhanced microbial stability, lower pH, improved acidification, and overall acceptance of the product. It can be concluded that probiotics active films are economically viable and offer a sustainable substitute for traditional cheese packaging, like parchment sheets, and are a promising packaging solution for the dairy industry that combines eco-friendliness with enhanced functionality.

Author contributions

Asghar Azizian collected the samples, conducted the experimental work, Negin Noori, Hassan Gandomi, Ali misaghi and Ali Khanjari nalyzed the data, writing and revising the manuscript.

Ethics

The authors of this study declare that all steps in this study, carried out in accordance with the principles of the ethics committee of the Iranian Veterinary Organization.

Conflicts of interest

There is no conflict of interest in the study.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgements

This work is based research funded by Iran National Science Foundation (INSF) under project No. 4013439

References

[1] Karimi R, Sohrabvandi S, Mortazavian AM. Sensory characteristics of probiotic cheese. Comprehensive Reviews in Food Science and Food Safety. 2012 Sep;11(5):437-52. [https://doi.org/10.1111/j.15414337.2012.00186.x]

[2] Pereda, M., Marcovich, N. E., & Mosiewicki, M. A. Sodium caseinate films containing linseed oil resin as oily modifier. Food Hydrocolloids. 2015; 44, 407-415. [https://doi.org/10.1016/j.foodhyd.2014.10.010]

[3] Han, J. H., & Aristippos, G. Edible films and coatings. A review. In Innovations in Food Packaging. 2005; 239–262). [http://doi.org/1016/10/B978-012311632-1/50047-4]

[4] Asikin Y, Taira I, Inafuku S, Sumi H, Sawamura M, Takara K, Wada K. Volatile aroma components and antioxidant activities of the flavedo peel extract of unripe Shiikuwasha (Citrus depressa Hayata). Journal of food science. 2012 Apr;77(4):C469-75. [https://doi.org/10.1111/j.1750-3841.2012.02648.x]

[5] Arafat, Y.; Altemimi, A.; Pratap-Singh, A.; Badwaik, L.S. Active Biodegradable Films Based on Sweet Lime Peel Residue and Its Effect on Quality of Fish Fillets. Polymers. 2021; 13, 1240. [https://doi.org/10.3390/polym13081240]

[6] Van Doan H, Hoseinifar SH, Naraballobh W, Jaturasitha S, Tongsiri S, Chitmanat C, Ringø E. Dietary inclusion of Orange peels derived pectin and *L. plantarum* for Nile tilapia (Oreochromis niloticus) cultured under indoor biofloc systems. Aquaculture. 2019 Jun 15;508:98-105. [https://doi.org/10.1016/j.aquaculture.2019.04.049]

[7] Mozaffarzogh M, Misaghi A, Shahbazi Y, Kamkar A. Evaluation of probiotic carboxymethyl cellulose-sodium caseinate films and their application in extending shelf life quality of fresh trout fillets. LWT. 2020 May 1;126:109305. [https://doi.org/10.1016/j.lwt.2020.109305]

[8] Soukoulis, C., & Bohn, T. A comprehensive overview on the micro- and nano-technological encapsulation advances for enhancing the chemical stability and bioavailability of carotenoids. Critical Reviews in Food Science and Nutrition. 2018; 58(1), 1–36. [https://doi.org/10.1080/10408398.2016.1181251]

[9] Ramos, M., Valdés, A., Beltrán, A., & Garrigós, M. C. Gelatin-based films and coatings for food packaging applications. Coatings. 2018; 6(4), 41. [https://doi.org/10.3390/coatings8040041]

[10] Burgain, J., Gaiani, C., Linder, M., & Scher, J. Encapsulation of probiotic living cells: From laboratory scale to industrial applications. Journal of Food Engineering. 2011; 104(4), 467–483. [https://doi.org/10.1016/j.jfoodeng.2011.03.010]

[11] Tripathi, M. K., & Giri, S. K. Probiotic functional foods: Survival of probiotics during processing and storage. Journal of Functional Foods. 2014; 9, 225–241. [https://doi.org/10.1016/j.jff.2014.06.007]

[12] Anal, A. K., & Singh, H. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends in Food Science & Technology. 2007; 18(5), 240–251. [https://doi.org/10.1016/j.tifs.2007.02.006]

[13] Arena, M. P., Silvain, A., Normanno, G., Grieco, F., Drider, D., Spano, G., & Fiocco, D. Use of *L. plantarum* strains as a bio-control strategy against food-borne pathogenic microorganisms. Frontiers in Microbiology. 2016; 7, 464. [https://doi.org/10.3389/fmicb.2016.00464]

[14] Figueroa-González, I., Quijano, G., Ramírez, G., & Cruz-Guerrero, A. Probiotics and prebiotics—Perspectives and challenges. Journal of the Science of Food and Agriculture. 2011; 91(8), 1341–1348. [https://doi.org/10.1002/jsfa.4379]
[15] Gbassi, G. K., & Vandamme, T. Probiotic encapsulation technology: From microencapsulation to release into the gut. Pharmaceutics. 2012; 4(1), 149–163. [https://doi.org/10.3390/pharmaceutics4010149]
[16] Silva, C. C. G., Silva, S. P. M., & Ribeiro, S. C. Application of bacteriocins and protective cultures in dairy food preservation. Frontiers in Microbiology. 2018; 9, 594. [https://doi.org/10.3389/fmicb.2018.00594]
[17] Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. International Journal of Food Microbiology. 2004; 94(3), 223–253. [https://doi.org/10.1016/j.ijfoodmicro.2004.03.022]
[18] Crowley, S., Mahony, J., & van Sinderen, D. Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. Trends in Food Science & Technology. 2013; 33(2), 93–109. [https://doi.org/10.1016/j.tifs.2013.07.002]
[19] Delgado, S., Rachid, C. T. C. C., Fernández, E., Rychlik, T., Alegría, Á., & Mayo, B. Diversity of the microbiota in traditional cheeses and challenges for food safety. Food Research International. 2019; 125, 108585. [https://doi.org/10.1016/j.foodres.2019.108585]
[20] Gálvez, A., Abriouel, H., López, R. L., & Ben Omar, N. Bacteriocin-based strategies for food biopreservation. International Journal of Food Microbiology. 2010; 120(1–2), 51–70. [https://doi.org/10.1016/j.ijfoodmicro.2007.10.028]
[21] Gomes, A. M. P., & Malcata, F. X. Bifidobacterium spp. and Lactobacillus spp. in dairy products: Microbiological, technological and probiotic aspects. International Dairy Journal. 1999; 9(11), 825–845. [https://doi.org/10.1016/S0958-6946(99)00130-2]
[22] Pereda, M., Añón, M. C., & Ruseckaite, R. A. Mechanical and barrier properties of edible films based on blends of sodium caseinate and chitosan. Food Hydrocolloids. 2012; 26(2), 167–175. [https://doi.org/10.1016/j.foodhyd.2011.09.020]
[23] Rhim, J.-W., Park, H. M., & Ha, C.-S. Bio-nanocomposites for food packaging applications. Progress in Polymer Science. 2013; 38(10–11), 1629–1652. [https://doi.org/10.1016/j.progpolymsci.2013.04.003]
[24] Sorrentino, A., Gorrasi, G., & Vittoria, V. Potential perspectives of bio-nanocomposites for food packaging applications. Trends in Food Science & Technology, 2007; 18(2), 84–95. [https://doi.org/10.1016/j.tifs.2006.11.006]
[25] Gobbetti, M., De Angelis, M., Di Cagno, R., Calasso, M., & Camarca, A. Pros and cons of probiotics in dairy products. International Dairy Journal. 2015; 42, 1–15. [https://doi.org/10.1016/j.idairyj.2014.10.013]