

Viability of *Lactobacillus Plantarum* Incorporated with Sourdough Powder-Based Edible Film in Set Yogurt and Subsequent Changes During Post Fermentation Storage

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ABSTRACT

Introduction: Due to the advantages of sourdough, its film production for food packaging could be interesting. This study aimed to evaluate the influence of probiotic sourdough based edible film covered on set yogurt and subsequent changes during post fermentation storage.

Materials and Methods: The parameters examined included changes to the fermentation characteristics (pH, and viable counts of probiotic bacteria), synergies, and sensory evaluation during 21-d storage at 4°C. *Lactobacillus plantarum* was supplemented with sourdough films and yogurt produced by commercial yogurt starters (*Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*) then films placed on yogurt containers, and a panel of parameters reflecting product quality was subsequently monitored along with 21-d post-fermentation storage.

Results: Results demonstrated that the pH value of yogurt decreased slowly during the storage and no significant difference was observed between the control and the samples with the films. Although the number of viable cells decreased during storage, it did not lower than the minimum requirement for probiotics ($> 10^7$ log CFU / g). The synergies of the film-treated samples were significantly ($P \leq 0.05$) lower than the control samples. The yogurt with the film without bacteria had the least synergy. Film-treated yogurts had acceptable sensory properties in comparison with control.

Conclusion: Sourdough films can be an optimizing candidate to enter the food industry as a bioactive edible film and also could improve the delivery of probiotic bacteria.

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Introduction

Today, research on probiotic films has expanded. These films are the result of the entrapment of probiotic bacteria in the film matrix. The original idea for these films was inspired by edible films that act as appropriate carriers for probiotic bacteria, so in the last decade, most studies have focused on the production and development of probiotic and antimicrobial films¹⁻

⁴. Most of the probiotics studied belongs to lactic acid bacteria (LAB)^{5, 6}. In LAB group, *Lactobacillus plantarum* is a very flexible and versatile species which has been marketed as a probiotic since 1999. This strain with the ability of adhering to human cells is generally found in many fermented food products including sauerkraut, pickles, brined olives, sourdough, and other fermented plant material⁷.

Due to probiotics health benefits, it is expected to account for a significant part of future research and market, but there are serious challenges are involved in reaching this horizon. One of the most important of these challenges is the viability of these bacteria in the film matrix. The viability of probiotics entrapped in edible films and coatings has contributed to specialized studies. Therefore, probiotics survival during the storage and gastrointestinal condition is considered a priority for researchers throughout the world^{8, 9}. Another challenge in the design and production of antimicrobial films is their structure and component¹⁰.

The compounds which are selected and utilized for antimicrobial films should be stable, accessible, and economically beneficial¹⁰. Sourdough is a biopolymer with unique properties that have the potential to face these challenges. This biopolymer is “a combination of whole wheat flour, salt, and water, which naturally is fermented by lactic acid bacteria and yeasts¹¹. Production of exopolysaccharide, acid, and microbial enzymes during sourdough fermentation and partial depolymerization of gluten macromolecules improve the viscoelastic properties of sourdough compared to wheat flour, starch, and gluten¹². Sourdough is a natural carrier for probiotic bacteria and contains several antimicrobial and functional substances¹³. Due to both antimicrobial and viscoelastic properties of sourdough, it is excellent polysaccharide-protein biopolymer to develop probiotic films.

Despite extensive research on films in food products, the application of films in yogurt has not been investigated according to the author's information. Yogurt is known as a product with many desirable effects for consumers, and in recent years, its popularity has increased significantly. Yogurt is a fermented milk product that has been

prepared by allowing milk to sour at 40–45 °C¹⁴. In some Greek-style and whole-milk yogurts, they are not homogenized because a thin cream layer on the top of yogurt is interested. The low permeability of this layer to oxygen decreases the growth of undesirable microorganisms and improves microaerophilic growth of starter bacteria resulting in more acid production. Therefore these kinds of yogurts are slightly more acidic than commercial yogurts^{1, 15}.

The protective layer on the surface of yogurt with antimicrobial and nutritional properties, as well as its barrier ability in limiting oxygen and penetrating spoilage agents, can improve the durability of this product^{13, 16}. In this study, the potential of probiotic sourdough film for improving yogurt quality was evaluated. For the first time, we inspired by the creamy layer on yogurt as an alternative to simulate a probiotic-based sourdough film. According to the literature review to date, no information is present considering the preparation of sourdough films and imitation of creamy layers of yogurt. This study aimed to fabricate, edible film from sourdough as a carrier for *Lactobacillus Plantarum* to imitate the creamy layer of yogurt for improving its quality. The findings of this research will contribute to create a bioactive edible film based on sourdough biopolymer and apply it in the production of yogurt, which improves the shelf life, sensory and nutritional properties of this product.

Materials and Methods

Preparation of sourdough powder

Whole wheat flour dough was allowed to ferment at 28 °C for 72 h. The fresh sourdough with a primary moisture content of 51.59% (wet basis) was dried and grounded at room temperature (Figure 1).



Figure 1: Sourdough powder

Preparation of films

The film suspensions (probiotic and control) were prepared by dispersing 10% sourdough powder in the distilled water and adding glycerol 10% level of the total dry matter under continuous stirring (400 rpm) at 25 °C. The film suspension was heated to 80 °C for 1 min for gelatinization of starch to increase the viscosity of the film suspension. Film solutions were cooled down to 40 °C. Afterwards, a *Lactobacillus plantarum* strain was used for the preparation of bioactive films.

Lyophilized culture of *Lactobacillus plantarum* subsp. *plantarum* PTCC 1745, isolated from pickled cabbage, was purchased from Iranian Research and Organization for Science and Technology. The microbial culture was regenerated by transferring 1 g of commercial preparation into 10 mL of De Man, Rogosa and Sharpe (MRS) broth (Merck KGaA, Darmstadt, Germany) and incubated at 37 °C overnight.

Activated cells were transferred to MRS agar (Merck KGaA, Darmstadt, Germany) and incubated in a plastic anaerobic jar with C type gas pack sachet (Merck KGaA, Darmstadt, Germany) at 37°C for 48 h. To being prepared a bacterial suspension the colonies were collected using a sterilized loop and diluted in the sterile distilled water. The colonies were adjusted to (10^9 CFU/mL) by Spectrophotometers - UV-Visible (Mecasys, Korea) to reaching a target inoculum.

Lactic acid bacteria were incorporated by adding the preparation of the bacterial cells into the suspension to reach 8 log CFU/g and homogenized. Following film inoculation, the number of viable microorganisms in the film suspension was determined. After wards, a certain volume of film dispersion (10 ml) was poured into petri dishes with 8 cm in diameter and held at 37 °C for 24 h. After drying, the films were separated from the plates and kept at 4 °C in the sterile plastic zip packs (Figure 2).

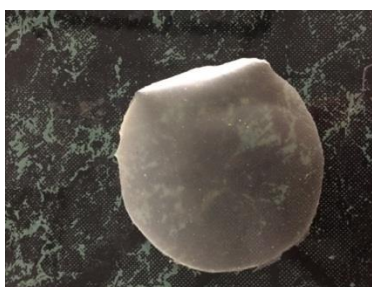


Figure 2: Edible film prepared by sourdough powder

Preparation of yogurt

Sterile and homogenized bovine milk with a fat content of 3.9% was heated at 85 °C. After cooling to 44 °C, it was inoculated with yogurt culture YZ-1 (micromilk S.R.I., Italia; 2 kg of batch starter/100 kg milk). The thoroughly mixed milk

was poured into containers (150 ml) and incubated at 44 °C for 4 -5 hours until the pH value reached 4.2. The yogurt samples were chilled at 4 °C (Figure 3). Then, their surface was covered with circular films and they were stored in the refrigerator for 21 days.



Figure 3: The yogurt samples containers

Properties of yogurt

The pH value of yogurts was determined in days 1, 7, 14 and 21 at 4 °C. Each yogurt sample (1 g) was mixed with distilled water (1:1, w/v) and pH was measured using a pH meter (Martini, Mi 151, china). Syneresis was determined using centrifugal methods¹⁷. The whey was measured following by placing 25 gr of yogurt on calibrated test-tube and centrifuged for 10 minutes by 1500 × g. The syneresis computed was expressed in %.

Enumeration of *L. plantarum*

The viability of *L. plantarum* entrapped in the sourdough film was tested during the storage time. Yogurt samples were covered with the films and evaluated for 21 days for applying probiotic and non-probiotic sourdough films in food model. For every test, 1 g of the film sample was mixed with 9 ml of sterile peptone water (1 g/L). After sequential dilutions, appropriate dilutions were plated on set MRS agar containing 10 mg/L of vancomycin. Then, they were incubated in a plastic anaerobic jar with C type gas pack sachet (Merck KGaA, Darmstadt, Germany) at 37 °C for 48 h. The total counts of the viable bacteria were reported as logarithmic colony forming units per gram (log CFU/g). Enumeration of the bacteria on agar plates using colony count technique. The total count of viable bacteria was expressed as log colony forming units per gram (log CFU/g, $\text{CFU/g} = \text{CFU/plate} \times \text{dilution factor}$).

Sensory Profiling

Seven panelists were chosen for sensory evaluation using qualitative descriptive analysis¹⁸. Each yogurt sample was coded using 3 random numbers. 4 main attributes including taste, texture in the mouth, and total acceptability was used to evaluate the taste. A 5 cm unstructured hedonic

scale were used in the total acceptability test with extremes of “1” extremely disliked and “5” extremely liked¹⁹. Sensory evaluation was done in 1, 7, 14, and 21 days.

Statistical analysis

All of the tests were carried out in triplicate or more replicates. Data reported as the mean values and standard deviation were used for statistical analysis. Statistical analysis of differences was carried out by one-way analysis of variance (ANOVA), and the means were compared using Duncan’s multiple range tests at $P < 0.05$. The software SPSS Inc., Chicago, IL, Ver. 21, was used to perform statistical analysis.

Results

Sourdough powder characteristics

The characteristics of obtained the sourdough powder were measured as follows: moisture ($8.02 \pm 0.40\%$), ash ($7.525 \pm 0.30\%$), protein ($11.42 \pm 0.10\%$), carbohydrate ($70.55 \pm 0.20\%$), Total Titrable Acidity (TTA) (11.00 ± 0.01), pH (4.30 ± 0.01), total count ($9.72 \pm 1.44 \log \text{CFU/g}$), and total lactobacilli ($5.22 \pm 1.33 \log \text{CFU/g}$).

pH value of yogurt

As shown in Figure 4, for all the yogurt treatments during the storage pH value was decreased. The pH change in both treated and control yogurt samples was similar in the 21st day of refrigerated storage. The pH of yogurts was found to be 4.40, 4.38 and 4.35 during the first day of storage and at the end of the storage period, the pH for control (yogurt without film) and yogurt with probiotic sourdough film and sourdough film was decreased to 3.97, 4.04, and 4.00, respectively. The data showed that no significant difference was observed between the control and the samples with the films.

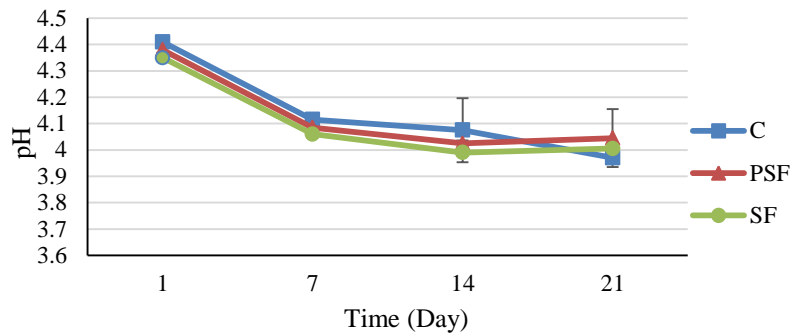


Figure 4: Effect of sourdough films on the pH value of yogurt samples covered with films and control, during storage at refrigerated (4 °C). PSF (probiotic sourdough film); SF (sourdough film) C (Plain yogurt).

Syneresis

Figure 5 indicates the syneresis rates in the storage period. The amount of syneresis of treated

samples (40.57-40.67%) was lower than that of the control (yogurt without film) (44.03%) at the end of 21st days of the storage.

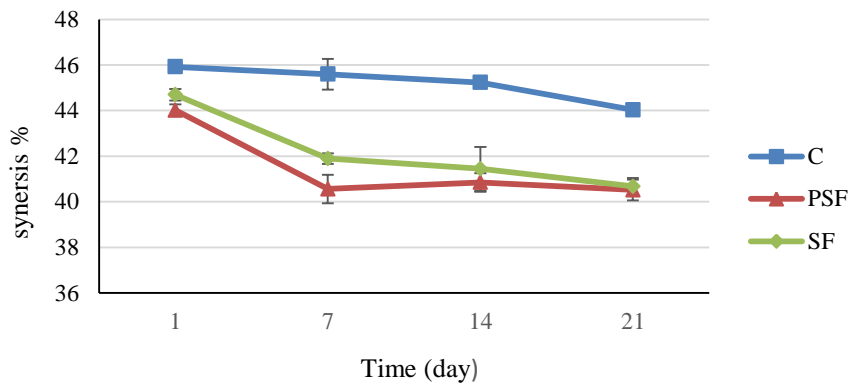


Figure 5: Effect of sourdough films on the syneresis of yogurt samples covered with films and control, during storage at refrigerated (4 °C). PSF (probiotic sourdough film); SF (sourdough film) C (Plain yogurt).

Viability of lactobacillus plantarum

In the food model (yogurt), the population of bacteria embedded in the sourdough film showed

significant ($P \leq 0.05$) decreases in the first 7 days (Figure 6). No significant decrease occurred on the days of 14 and 21.

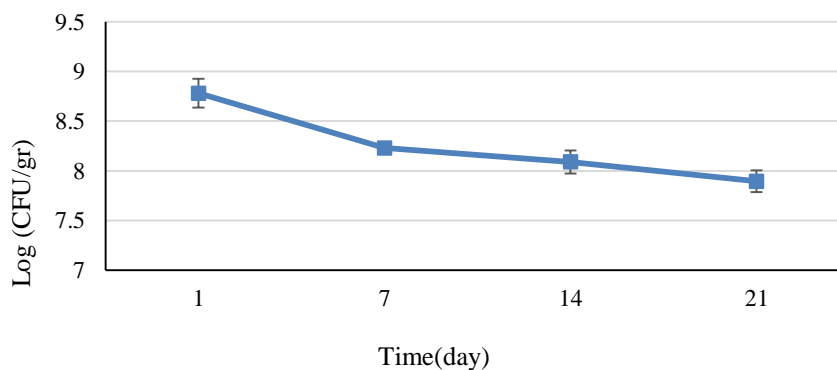


Figure 6: Viability of *L. plantarum* entrapped in sourdough under food model (yogurt) condition, during storage at refrigerated (4 °C).

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Sensorial assessment

Figure 7 shows the sensory scores of the yogurt samples. The results of the sensory tests showed that consumers ranked the sample containing sourdough film. However, on

the 7th day, the control sample had a higher score as compared to the film-treated samples. But on the 14th and 21st days, film-treated yogurt samples gained more score and acceptability.

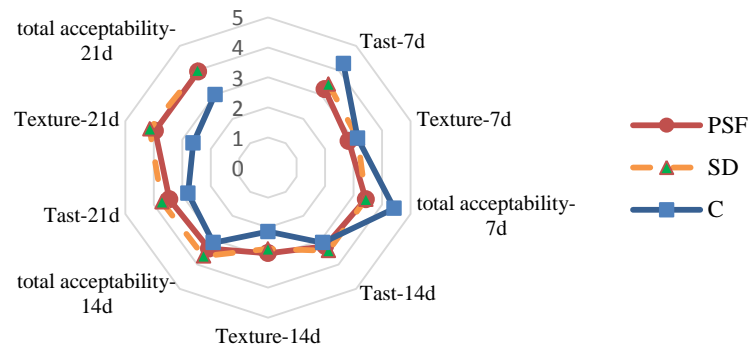


Figure 7: Sensory evaluation of yogurt samples treated with sourdough films and control during storage at refrigerated (4 °C). C (Plain yogurt), PSF (yogurt with probiotic sourdough film), SF (yogurt with sourdough film).

Discussion

Probiotic bacteria must survive at the time of consumption and the digestive tract and reach the colon in large quantities²⁰. An attempt has been made herein to examine the viability of *L. plantarum* incorporated with sourdough-based film and the effectiveness of sourdough films in yogurt quality. In our study, the sourdough powder was incorporated at a concentration of 10% (w/v). Furthermore, for the first time, we inspired by the creamy layer on yogurt as an alternative to simulate a probiotic-based sourdough film. According to the literature review to date, no information is present considering the preparation of sourdough films.

Control and film-treated yogurts showed a similar pH trend during the storage period at 4 °C for 3 weeks (Figure 4). These results were confirmed by previous results of Ortakci who reported that free and encapsulated probiotic *Lactobacillus acidophilus* in yogurts followed a similar pH trend during the storage period at 4 °C for 4 week⁴. As well, Ziar et al.²¹ reported that the final pH in yogurts contains free and encapsulated *Bifidobacterium animalis subsp. lactis Bb12* and

Lactobacillus rhamnosus were similar at the end of the storage period (28 days) at 4 °C. Bacteria trapped in the film matrix appear to prevent the release of the acid produced by them. This phenomenon can solve the problem of increasing the effect of free probiotics in causing organoleptic changes, while this film can also improve the nutritional properties of yogurt.

At the presence of film, more water absorption occurred in yogurt samples, and therefore the syneresis rates were decreased significantly ($P > 0.05$) compared to plain yogurt (Figure 5). Syneresis rate which is defined as whey separation is a common problem in yogurt and a high level of it indicates the low quality of the product^{22, 23}. Thus, the presence of the film on the yogurt surface reduces this defect.

As mentioned, sourdough is a community that comprises bacteria, yeasts and fungi. In this ecosystem, microorganisms can enrich the sourdough with several metabolites including organic acids, exopolysaccharides, antimicrobial compounds, and enzymes that improve bacterial survival conditions. In fact, the sourdough film suspension can be considered as a free cell

suspension, because to prepare the film, it was heated to 80°C to kill all its vegetative cells, and the results of the total count of film suspension before probiotics inoculation, supported this claim (the data not shown). On the other hand, the presence of bacteria exopolysaccharides in sourdough could create a network that entraps the probiotics. Accordingly, as expected, these conditions can provide a high level of protection and minimize the tensions. The enhancement in bacterial survival in yogurt is an attractive aspect for any commercial exploitation because bacteria will remain intact in the food until consumption. The effects of bacterial survival in yogurt largely depend on the method of film preparation and casting or microencapsulation. In this study, entrapment in the sourdough matrix was found to improve the viability and to maintain a suitable post-acidifying activity of beneficial organisms in yogurt after 21 days of storage at 4°C reinforcing the valuable role of sourdough for efficient protection of probiotics.

As shown in figure 6 the number of viable bacteria decreased significantly in 7 days. These results corresponded with previous studies on microencapsulated probiotic bacteria in yogurt²⁴. Due to the high moisture content of yogurt, the reason for this is osmotic shock during rehydration. Rehydration of probiotics is the critical step for the revival of cells after dehydration. The rehydration media also can influence probiotic recovery significantly^{13, 25}. Since yogurt is a nutritional environment with the appropriate pH for probiotics survival, they were able to recover, which led to a stability increase in the number of bacteria until the end of storage (21 days). According to the results, *L. plantarum* in the films applied in yogurt samples presented good cell viability. Losses of the bacterial population were only 0.89 log cycles.

Among the various criteria that are taken into account for novel food product development, sensory quality (i.e., customer acceptability and preferences) should be of prime consideration²⁶. Sensory quality can be rated by the flavor, texture, and appearance²⁷, and no adverse effect on the taste or aroma of the products should be introduced

when probiotics or adjunct cultures are to be applied²⁸. Our results show that the addition of any of the tested films did not exert a negative effect on the product's sensory quality and also, the overall quality of film-treated yogurts was evaluated well by panelists (Figure 7). It seems that the presence of films on the surface of yogurt has maintained the freshness and desirable organoleptic properties. Although film-containing samples gained more points regarding the panelists, there was no significant difference between the samples with probiotic sourdough film and sourdough film. As previously explained, bacteria entrapped in the film matrix could harm the organoleptic properties of yogurt. In this regard, Adhikari and Değirmenci reported that probiotic bacteria could increase yogurt acidity without being encapsulated^{22, 29}. It also could reduce consumer acceptance of yogurt. During the fermentation or post-fermentation storage of yogurt, probiotics or adjunct cultures could cause many complex physical and chemical changes, such as proteolysis and acidification, which involves the gradual casein hydrolysis to form polypeptides, AA, and other compounds³⁰. Thus with entrapment of probiotics in an appropriate matrix could be prevented these undesirable changes.

Conclusion

Sourdough, as a unique polymer source, has an excellent potential to develop edible films. According to the results of this study, sourdough films can improve the quality and nutritional properties of food products at the same time. The sourdough film had sufficient barrier ability as a food packaging. In this study, the film had favorable effects on the sensory properties of yogurt samples. There is no significant difference was observed in pH between the control and the samples with the films. Therefore, based on the results of this research, sourdough films can be an optimizing candidate to enter in the food industry as a bioactive edible film. However, complementary research is needed to evaluate its application in other food products.

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Conflict of interest

The authors declare no conflict of interests.

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