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Edible Films Incorporating with *Lactobacillus plantarum* Based on Sourdough, Wheat Flour, and Gelatin: Films Characterization and Cell Viability During Storage and Simulated Gastrointestinal Condition

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Incorporating probiotic bacteria with different biopolymers as edible films is an effective approach to improve their viability. In this study, three different films (wheat sourdough powder, whole wheat flour, and bovine bone gelatin) are incorporated with *Lactobacillus plantarum* separately and the viability of bacteria is monitored during the storage time (40 days at 4 °C) and simulated gastrointestinal conditions. The results demonstrated that the sourdough film has the best protective effect on the viability of the cells during the film's preparation, storage time (6.5 Log/CFU/g), and simulated gastrointestinal conditions (7.13 Log/CFU/g). A higher rate of reduction is observed in gelatin film at the end of the storage time (4.03 Log/CFU/g). Physicochemical, and mechanical characteristics are examined. It is observed that the incorporation of bacteria does not affect the thickness, moisture content, and solubility of all films, but changed the mechanical properties of the sourdough and the wheat flour films ($p \leq 0.05$). Scanning Electron Microscope images showed a more uniform and compact structure for both bacterial and control gelatin films. Although the sourdough film is appropriate for protecting probiotic bacteria (>10^6 CFU/g), further studies are needed to improve its mechanical properties.

1. Introduction

The global probiotics market size is a good indication of the unprecedented growth of these products in the world. According to the study provided in Markets and Markets (2019), forecasts report that the global market will reach a total of $69.3 billion by 2023.[1] This growing demand for probiotic foods has aroused the attention of food industries and researchers and persuaded them to investigate new food products attributed by probiotics.

The most eminent characteristic of probiotics is the maintenance of the viability and the metabolic activity throughout the food processing and consumption times. The first generation of probiotics was presented directly to food products in the form of lyophilized planktonic bacteria. This method reduced the viable bacteria during food storage and processing. Also, bacterial activity had a detrimental effect on the organoleptic properties of the food products. The second generation was introduced in order to improve the viability of probiotic bacteria, as well as to promote organoleptic properties of food products.[2] In this generation, probiotics were entrapped in natural or synthetic polymers.[3] The problem with this technique was its low release ability. In order to solve this problem, microencapsulation and composite polymers techniques were created the third generation of probiotics. In this generation, bacteria were entrapped in polymeric materials by mechanical or physicochemical processes such as extrusion, emulsification, and spray-drying.[4] The major problem with these methods is high cost, needs for new technologies, and the failure to resolve the problem of survivability. Research has indicated that the formation of biofilm by bacteria can be an appropriate protection mechanism. Biofilm is a complex ecosystem that included bacteria and their metabolites. This is the main idea for the introduction of the fourth generation of probiotics. In the fourth generation, bacteria are protected by metabolites they produce themselves.[5]

In the last decade, a great number of studies have focused on the production and development of probiotic and antimicrobial films.[6–9] Wheat sourdough is a unique biopolymer for film formation, which can be very efficient in probiotics maintenance. Sourdough is "a combination of whole wheat flour, salt, and water, which is fermented naturally by lactic acid bacteria and yeasts.[10] Sourdough has exopolysaccharide, acid, and microbial enzymes, which are produced during fermentation. Fermentation causes the macromolecule of gluten to be partially depolymerized, thus sourdough has better viscoelastic properties than wheat flour, starch, and gluten.[11] Sourdough is known as a natural carrier for probiotic bacteria and contains varieties of antimicrobial and functional substances.[12] Accordingly, sourdough could be an outstanding polysaccharide-protein biopolymer for developing probiotic films. To the best of our knowledge,

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the literature has not provided significant information about the preparation process of Sourdough films.

To investigate the differences between sourdough and wheat flour, and also to understand the impact of fermentation in the properties of films, wheat flour was studied as a supplementary film source. Wheat flour could be considered a highly available, low-cost, and nutritious substance, and contains a complex combination of carbohydrates and protein. The presence of gluten as a protein in wheat flour causes the desired deal of elasticity and adhesion.[13] Based on past studies, the oxygen permeability rates of the wheat flour film are low enough such that when fresh-cut fruits are covered with a wheat flour film, respiration is minimized and as a result, prevents them from being rotten. Furthermore, wheat flour films are transparent and odorless, and as a result, they do not affect the flavor and properties of the products.[14,15] According to the mentioned conditions, the wheat flour can be an appropriate starch-protein biopolymer to develop the edible films.[16]

To compare wheat flour and sourdough films with other typical films, this study also examines bovine bone gelatin which is a well-known film source in the literature of this research. Gelatin has long been popular in the literature due to the possession of a set of desirable properties such as its film-forming capabilities, the provision of an outer barrier to protect food from drying, its resistance when exposed to oxygen and light, and some other desirable functional properties.[17] Gelatin films exhibit significant mechanical properties, although being sensitive to moisture and present weak barrier attributes against water vapor. All in all, sourdough, wheat flour, and gelatin could investigate as appropriate biopolymers for the development of probiotic edible films.

Most of probiotic bacteria are a sub-category of lactic acid bacteria (LAB) group.[18,19]. In the LAB group, *L. plantarum* is a very flexible and versatile type species that has been marketed as a probiotic since 1999. This strain with the ability to adhere to human cells is generally found in many fermented food products.[20]

The objective of this study was to prepare the edible films based on wheat sourdough, wheat flour, and bovine bone gelatin, with the addition of *L. plantarum*. The physical, mechanical and, structural properties of the resulted films were investigated. Moreover, the relative viability of probiotic strain was examined in storage and simulated gastrointestinal conditions. The findings of this research will contribute to creating a bioactive edible film based on biopolymers which can improve the shelf life, and nutritional properties of the food products.

2. Experimental Section

2.1. Preparation of Sourdough Powder

A whole wheat flour (1000 g) (11% moisture, 13.2% protein, and 0.43% ash), water (900 mL) and salt (20g) were mixed. Then, wheat flour dough was allowed to ferment at 25 °C for 72 h (in a single stage). The fresh sourdough with a primary moisture content of 51.59% (wet basis) was dried and grounded at room temperature. The characteristics of the obtained sourdough powder were reported as follows: moisture (8.02 ± 0.40%), ash (7.525 ± 0.30%), protein (11.42 ± 0.10%), carbohydrate (70.55 ± 0.20%), Total Titrable Acidity (TTA) (11.00 ± 0.01), pH (4.30 ± 0.01), total count (9.72 ± 1.44 log CFU/g), and total lactobacilli (5.22 ± 1.33 log CFU/g).

2.2. Preparation of Bacterial Incorporated Edible Films

Lyophilized culture of *L. plantarum* subsp. *plantarum* PTCC 1745, isolated from pickled cabbage, was obtained from Iranian Research Organization for Science and Technology. The microbial culture was activated according to the company's instruction. The activated bacteria were transferred into De Man, Rogosa, and Sharpe agar (MRS) (Merck KGA, Germany) and incubated under anaerobic conditions at 37 °C for 48 h. The colonies were collected with a sterilized loop and suspended in the sterile distilled water. The bacterial suspension were adjusted to (10^9 CFU mL^{-1}) by Spectrophotometers—UV-Visible (Mecasys, Korea) to reaching a target inoculum.[21] The aqueous suspension of films contained 10 g of sourdough powder, wheat flour, and bovine bone gelatin (food grade) which were prepared separately by dissolving each in 100 mL of distilled water while stirring with a magnetic stirrer at room temperature. Following this, glycerol (1 g) as the plasticizer, was added to each film solution. Subsequently, all the solutions were heated at 80 °C for 1 min. After cooling to 40 °C, 10 mL of bacterial suspension was directly inoculated and get homogenized. Afterward, a certain volume of film dispersion (10 mL) was poured into Petri dishes with 8 cm in diameter and allowed to dry at 37 °C for 24 h. After drying, the films were separated from the plates and kept at 4 °C in sterile plastic zip packs. The aforementioned procedure was implemented without the incorporation of probiotic cells (which are normally used to prepare the control film samples).

2.3. Enumeration of *L. plantarum* in Films

The viability of *L. plantarum* entrapped in the films, was tested before the drying and storage period (40 days) at 4 °C. For each test, 1 g of the film samples (1 mL of each film solution for enumeration cells before drying) was mixed with 9 mL of sterile peptone water (1 g L^{-1}). After sequential dilutions, appropriate dilutions were plated on set MRS. 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). All the experiments were performed in triplicate which means that each experiment was repeated at least for three times. The survival rate of the bacteria during the drying process was calculated according to the following equation.[22]

$$\%\text{viability} = (N/N0) \times 100 \quad (1)$$

Where, (N_0) is the number of viable bacteria before the drying process and (N) represents the number of viable bacteria after the implemented drying process.

Enumeration of the bacteria on agar plates was performed using colony count technique.[23] The total number of viable bacteria was obtained by taking the log of the colony forming units per gram (log CFU/g, CFU/g = CFU/plate × dilution factor).

2.4. Viability in Simulated Gastrointestinal (SGI) Condition

The viability of *L. plantarum* into (SGI) conditions was examined using a procedure introduced by Krasaekoopt et al. (2004) with a set of small modifications.[24] At first, 1 g of the bacterial films and 3 mL (10^8 CFU) of the suspension containing free cells of *L. plantarum*, as a control, were separately added into the test tubes containing 30 mL of gastric juice. The gastric juice was prepared by sodium chloride (2.00 g L^{-1}), potassium chloride (1.12 g L^{-1}), potassium phosphate monobasic (0.40 g L^{-1}) and calcium chloride (0.11 g L^{-1}). The prepared solution was sterilized at 121 °C for 15 min. 0.26 g L^{-1} of porcine gastric mucosa powder (Sigma-Aldrich, USA) was added to gastric juice immediately before testing, and the pH was adjusted from 1.2 to 3.0 by adding 1 N HCl. All the tubes were incubated at 37 °C in agitation conditions. Viable bacteria were cultured at the following minutes of exposure: 0, 30, 60, and 120. In the next step, the porcine pancreatin (1.95 g L^{-1}), and bovine bile salt (0.18 g L^{-1}) (Sigma-Aldrich, USA) was added to the all tubes from the previous stage, then, pH was adjusted to 7.0 by adding 1 N $NaHCO_3$. The tubes were kept in the incubator in the shaking condition, and viable bacteria were cultured after each hour for 4h (60, 120, 180, and 240 min). All the experiments were carried out in triplicate.

2.5. Film Characteristics

2.5.1. Thickness

Digital micrometer (Mitutoyo No.293-766, Tokyo, Japan) with an accuracy of 1 µm was used to measure the thickness of sourdough films. For this purpose, the thickness of each sample was recorded at least at 10 random positions. The thickness values were used to calculate WVP (water vapor permeability) and tensile properties.

2.5.2. Moisture Content

The moisture content of each film sample was measured according to Ebrahimi et al. (2017).[7] To evaluate the moisture content, the films were dried in the laboratory oven at 105 °C until the weight was constant. The samples were weighted with an accuracy of 0.001 g before and after the drying process. The results were reported as grams of water per 100 g. Three replicates from each sample were used to perform these measurements. The weight loss of the samples was calculated using the following equation.

$$\%MC = (Mi - Mf)\,/Mi \times 100 \quad (2)$$

Where, (M_i) and (M_f) are the masses of primary and dried samples, respectively.

2.5.3. Solubility in Water

The water solubility of films was evaluated according to a method introduced by Tongdeesoontorn et al. (2012) with a set of minor modifications.[25] The film samples were cut (3 cm × 3 cm) and dried in a laboratory oven (105 °C for 5 h). Each film was submerged into a beaker containing 30 mL distilled water. Following the film samples for 24 h at 25 °C under shaking condition, the film residual were removed by filtering and drying in the oven at 105 °C for 5 h. Three replicates were measured for each sample. The percentage of soluble matter was calculated based on the following formula.

$$\%WS = (WI - WF)\,/WI \times 100 \quad (3)$$

where, (W_I) and (W_F) represent the primary weight and final weight, of the dried undissolved film, respectively.

2.5.4. Water Vapor Permeability (WVP)

The WVP of films was determined gravimetrically according to the ASTM E96-00 method.[26] The film samples were placed on the circular test cups containing 3.0 g anhydrous sodium chloride (0% RH, assay cup) and sealed. The effective area of the film was 31.4 mm^2. All these cups were maintained in a desiccator which contained a saturated solution of sodium chloride at 25 °C. The cups weight was measured with an accuracy of 0.001 g during 3 h intervals for 48 h. All the experiments were performed in triplicate. WVP was calculated based on the following formula.

$$WVP = (\Delta m/\Delta tA)\,.\,(X/\Delta P) \quad (4)$$

where (A) is the area of exposed film surface (m^2), $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/s), X is film thickness (m), and Δp is the difference between the water vapor pressure values of the two films.

2.5.5. Mechanical Properties

In this study, tensile strength (TE) and Elongation at Break (EAB) were determined as mechanical quality parameters. The mechanical qualities of the films were determined at 25 °C and 50% RH. The D882-18 standard test method and testing instrument of H5KS Stable Micro System, UK, were used in this study.[27] The films were placed under the conditions of 50% relative humidity in a desiccator containing saturated solutions of Mg $(NO_3)_2$ for 48 h and cut into rectangular strips (20 mm × 100 mm). The film strips were placed between the grips of the testing instrument. The primary grip distance and the cross-head speed were set at 50 mm and 5 mm min^{-1}, respectively. Three replicates from each sample were used to perform these measurements. The following formula was used to calculate the tensile strength and the elongation at break.

$$TE = F/A \quad (5)$$

$$\%EAB = ((L2 - L1)\,/L1)\,.100 \quad (6)$$

Force (F) represents the area of film surface (A), (L_1) and (L_2) are the primary and current length of the film, respectively.

2.5.6. Microstructural Properties

The Films microstructure was evaluated by Scanning Electron Microscopy SEM (EM-3200, KYKY, China). The films were broken in liquid nitrogen and fixed on aluminum stubs with double-sided adhesive tape. After that, the films were covered with a thin layer of gold using a BAL-TEC SCD 005 sputter coater (BALTEC AG, Balzers, Liechtenstein). SEM imaging was prepared at low pressure and an accelerating voltage at 20 kV.

2.6. Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectrum of absorption or emission of the matter was measured using Fourier Transform Infrared Spectroscopy (FTIR) (Thermo Nicolet, Avatar 370, USA). To prepare the discs, the films were initially powdered, and at the next step, approximately 2 mg of the film's powder was blended thoroughly with 70 mg of spectroscopic grade KBr. Afterwards, the powder was pressed into the pellets to obtain a clear transparent disc with 15 mm in diameter and 0.54 mm in thickness. All FTIR spectra were represented in the mid-infrared range (400–4000 cm^{-1}) at 25 °C. Typically, 32 scans were signal-averaged for a single spectrum at a spectral resolution of 4 cm^{-1}. To minimize the difficulties arising from unavoidable shifts, the entire spectrum was baseline-corrected in the region of 4000–400 cm^{-1}.

2.7. Statistical Analysis

All the tests were carried out in triplicate or more replicates. The mean values and the standard deviation were used to report the results in our statistical analysis. The One-way analysis of variance (ANOVA) was used to provide our statistical analysis, and the means were compared using Duncan's multiple range test at $p < 0.05$. The software SPSS Inc., Chicago, IL, Ver. 21, was used to perform statistical analysis.

3. Results and Discussion

3.1. Viability During the Drying Process

One of the major critical points in the survival of probiotics is the film casting and drying process. The ability of the film matrix to protect bacteria during the drying process is an important criterion for evaluating probiotic films. The viable counts of *L. plantarun* before and after the drying process are shown in **Figure 1** reports that among the incorporated films, sourdough has a better bacteria protection against shocks incurred during the film preparation process.

The number of bacteria has almost remained unchanged (99%) in sourdough film. Note that, sourdough is a community comprising bacteria, yeasts and fungi. In such an ecosystem, microorganisms can enrich the sourdough with several metabolites such as organic acids, exopolysaccharides, antimicrobial compounds, and enzymes that enhance 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

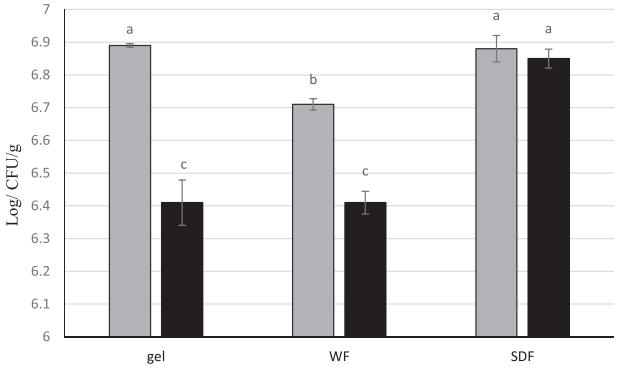


Figure 1. Survival of *L. plantarum* throughout drying at 37 °C for film obtaining. The numbers of viable microorganisms in film-forming dispersion are presented in light grey and the corresponding number in obtained films in black. Different letters indicate significant differences between films and within each film ($p \leq 0.05$). Gel, gelatin film; SDF, sourdough film; WF, wheat flour film

to 80 °C. The number of bacteria before probiotic incubation process shows that, this eliminates all the vegetative cells (the data not shown). The presence of bacteria exopolysaccharides in sourdough could create a network that entraps the probiotics.[28] Accordingly, as expected, these conditions can provide a high level of protection and minimize the tension arising from drying.

Due to the high water holding capacity of gelatin and gluten, as well as the formation of the jelly structure, gelatin and wheat flour films were able to prevent from the stress of dehydration during drying presses.[29] Piermaria et al, (2015) reported that the survival of *L. plantarum* in the kefiran film matrix during the drying period was 63%.[30] Ebrahimi et al, (2018) reported that no significant reduction in the viability of probiotics (*Lactobacillus acidophilus*, *L. casei*, *L. rhamnosus*, and *Bifidobacterium bifidum*) was observed during the drying process in the carboxymethyl cellulose film matrix.[7] Although the bacterial protection performance was high for wheat flour and gelatin films, 5 to 7% of the bacteria were reduced during the drying. The study of Fu & Chen, (2011) explains this observation as since the drying temperature is lower than the level which denatures the essential components of the cells, cell membrane damages may have led to the dehydration and inactivation of bacteria. During the drying process, starvation and other natural reasons such as oxidative or temperature stress increasing intracellular pH, capillary forces, and salt concentration could affect the survival of microorganisms.[31] The sourdough film seemed to be able to provide a good protection level of probiotic during the drying process.

3.2. Viability During the Storage Time

The viability of *L. plantarum* was correlated with storage (**Figure 2**). As expected, the number of viable probiotic cells decreased in all the film samples ($p < 0.05$) at the end of the storage period. As can be seen in Figure 2, gelatin and wheat flour films, the viability of *L. plantarum* decreased by 3.3 and 3.24 log/CFU after 40 days, respectively. While in the sourdough film, the reduction in the number of bacteria was only 0.56 log/CFU. This result is consistent with past studies reported on microencapsulated probiotic bacteria in yogurt,[32] and fibers

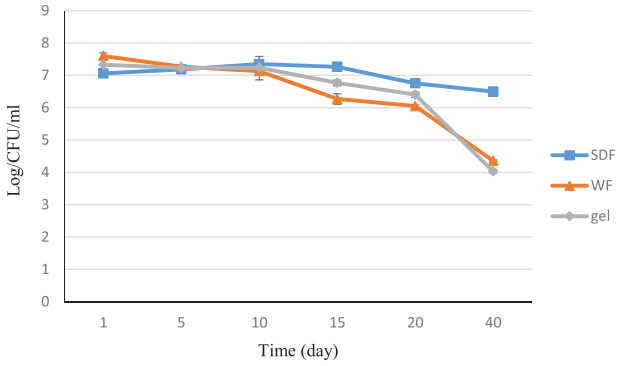


Figure 2. Number of viable *L. plantarum* (expressed in log CFU mL^{-1}) in the films over time (days). Sourdough film (SDF) represented solid line, wheat flour film (WF) represented by the round dot line and gelatin film (gel) represented by the dash line. Error bars indicate significant differences between films and within each film ($p \leq 0.05$).

(inulin, polydextrose, glucose-oligosaccharides and wheat dextrin) as prebiotic co-components of gelatin-based matrices.[33]

In all the film samples, the presence of adequate nutrients and water content in the film matrix provided a suitable environment for the bacterial growth, together with a low temperature reduces the activities of microorganisms. However, in the sourdough film, the presence of starch, protein, and microorganisms metabolite could lead to an enhanced protection of the probiotic cells.

3.3. Viability in Simulation Gastrointestinal Condition

Figure 3 shows the viability of *L. plantarum* in the films during sequential exposure to simulated gastric juice (SGJ, pH 3.0) for 120 min. (A). Simulated intestinal juice (SIJ, pH 7.0) for 240 min (B). In gastric conditions, the free cells of *L. plantarum* were decreased by 3.55 log/CFU after a 30 min exposure to acidic conditions. Whilst, the declining value for probiotic bacteria of the films matrix was found to be 2.25, 2.87, and 4.79 log/CFU in the same condition for sourdough, wheat flour and gelatin respectively (Figure 3A). Free cells did not survive (detectable level was 10^2 CFU g^{-1}) after a 60-min exposure. This data is consistent with the Ortakci and Sert, (2012) report who encapsulated *L. acidophilus* in calcium alginate.[34] The viability rate of *l. plantarum* entrapped in the sourdough film after 2 h incubation in simulated gastric juice was 74.37% (6.15 Log CFU/g). The wheat flour film had a behavior which was similar to the sourdough film, while this data for the gelatin film was 43.64% (3.71 Log CFU/g). It seems that because the gelatin film exhibits more water solubility, it releases the bacteria and as a result increases the exposure to the acid. The beneficiary effects of probiotic bacteria are associated with their survival through the human gastrointestinal tract. Bacterial death occurs along with the whole gastrointestinal (GI) tract. The acidic condition of the stomach and the existence of bile into the duodenum are the main factors affecting the bacteria survival. Therefore, a significant issue is that the probiotics must remain viable in the harsh conditions of the GI tract and should be able to reach the large intestine.[35] Most bacteria are equipped with a physical or chemical barrier to resist acid exposure. For example, *Bifidobacterium breve* NCIMB 8807 is protected by a polymer matrix containing ethylcellulose against acid treatment.[36] The viable counts of *L. plantarum* encapsulated by modifying starches were significantly higher than free cells, and remained more than 10^7 CFU g^{-1} after 2 h exposure to acid.[37]

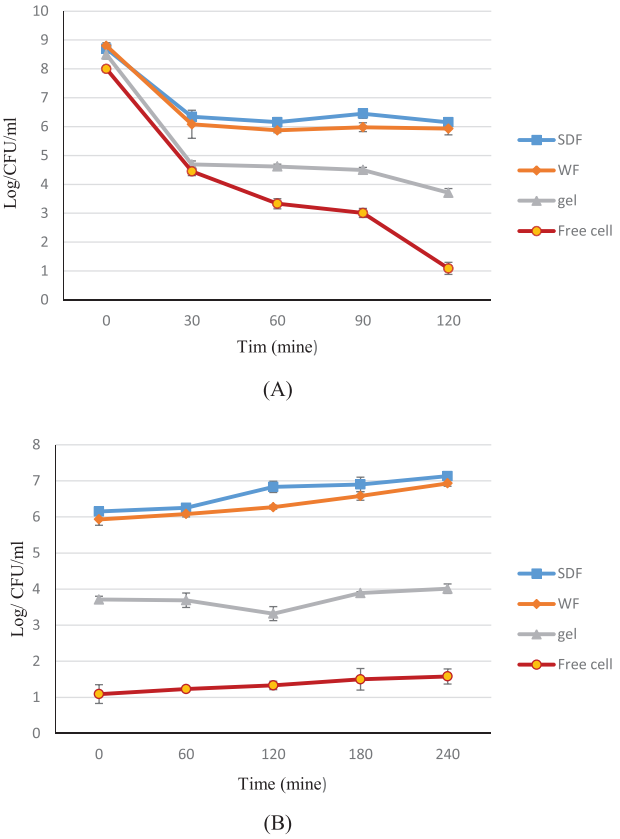


Figure 3. Viability of *L. plantarum* in the films during sequential exposure to simulated gastric juice (SGJ, pH 3.0) (A). Simulated intestinal juice (SIJ, pH 7.0) (B) for 240 min. Gel, gelatin film; SDF, sourdough film; WF, wheat flour film. Error bars indicate significant differences between films and within each film ($p \leq 0.05$).

Past studies report that free cells of *L. plantarum* showed high resistance against the bile treatment and after one hour of bile exposure, no reduction in the numbers of viable cells was observed.[29]

As shown in viable cells in the free and the entrapped sets, were constant between the first and the third hour of the experiment. While, the number of entrapped cells significantly increased during the fourth hour. The increase in the number of cells was greater in wheat flour and Sourdough than the gelatin film (Figure 3B).

This observation is in line with Gagliarini, et al., (2019) report.[38] In the mentioned study, whey protein-kefiran films as a carrier for *Lactobacillus paracasei CIDCA 8339* and *Kluyveromyces marxianus CIDCA 8154* to the gut, were investigated. It seems that live bacteria, after passing through the stomach and entering the neutral environment of the intestine, were able to recover.[39] In the present study, cell viability, after a sequential treatment, indicated that the sourdough and the wheat flour films provided a proper protection for probiotics in (GI) condition.

Table 1. Physical and mechanical properties of the films.

Film	Thickness [μm]	MC,%	WS,%	WVP × 10^{-7} [g. $m^{-1}.h^{-1}.pa^{-1}$]	TS [Mpa]	EAB, %
PSDF	273.33 ± 20.51^{a}	7.40 ± 0.50^{b}	25.38 ± 2.60^{c}	3.30 ± 0.08^{a}	1.91 ± 0.01^{d}	95.10 ± 10.90^{a}
SDF	240.00 ± 18.25^{a}	5.90 ± 1.08^{b}	22.38 ± 1.90^{c}	1.44 ± 0.08^{b}	3.64 ± 0.10^{c}	44.25 ± 8.90^{b}
PWF	267.50 ± 19.09^{a}	6.23 ± 0.4^{b}	35.05 ± 1.06^{b}	1.46 ± 0.05^{b}	5.91 ± 0.7^{b}	77.1 ± 11.1^{a}
WF	282.50 ± 19.08^{a}	5.29 ± 0.5^{b}	32.95 ± 0.2^{b}	1.38 ± 0.02^{b}	7.32 ± 0.5^{a}	43.69 ± 5.7^{b}
PGF	156.00 ± 28.72^{b}	7.93 ± 0.20^{a}	43.52 ± 1.25^{a}	1.58 ± 0.09^{b}	5.24 ± 0.35^{b}	75.90 ± 11.60^{a}
Gel	170.00 ± 26.35^{b}	7.78 ± 0.02^{a}	41.62 ± 1.65^{a}	1.7 ± 0.18^{b}	5.66 ± 0.61^{b}	71.55 ± 16.73^{a}

EAB, elongation at break; gel, gelatin Film, MC, moisture content; PGF, probiotic gelatin film; PSDF, probiotic sourdough film; PWF, probiotic wheat flour film; SFD, sourdough film; TS, tensile strength; WF, wheat flour film; WS, water solubility; WVP, water vapor permeability. Different letters in the same column indicate significant differences among formulations ($p < 0.05$). Values are means of three replicates ± standard deviation.

3.4. Physical Properties

3.4.1. Thickness

As shown in **Table 1**, the thicknesses of the gelatin films significantly varied from the sourdough and the wheat flour films. The minimum measured thickness was belonged to the gelatin film and the maximum belonged to the wheat flour films. Also, the results indicated that, no significant difference was observed between the thickness of the control and the probiotic films at the confidence level of 95%. The results of this study were consistent with those reported by Soukoulis et al. (2014) and Pereira et al (2016),[33–41] in the mentioned research probiotic bacteria (*Lactobacillus rhamnosus GG*) changed the thickness of the films (sodium alginate or sodium alginate and whey protein). Controlling the films' thickness is an important task since it can affect the mechanical, barrier, and transparency characteristics of the films.[40] Generally, the thickness of edible films should be less than 300 μm.[39] In this study, both films, either with or without probiotic bacteria, were qualified as edible films in terms of thickness. In the case of probiotic films, the thickness is important for protecting the microorganisms.[41] Gelatin based-films had a lower thickness value than the sourdough and the wheat flour films ($p < 0.05$) which may explain the greater survivability of probiotic cells in the sourdough and the wheat flour films. Variation in the film thickness could depend on the type of the incorporated solid matter as well as the amount. Moreover, the preparation method and drying conditions also, can affect the film thickness [43-7].

3.4.2. Moisture Content

The moisture content of the films which was prepared with the gelatin, the sourdough, and the wheat flour are shown in Table 1. The results of the moisture content were from 5.29% to 7.93%. The gelatin films were found to have higher moisture content (7.87% and 7.93%, respectively for control and bacterial films) than the other films. In all cases, there was no difference ($p < 0.05$) between the control and the bacterial films. The post drying moisture content is an important parameter that influences the rate of probiotics viability during the storage period.[43] The optimum moisture content for edible films is recognized to be 5–8%.[45] Therefore, the edible films in the present study contained an optimum moisture content. Our results are similar to the obtained by Piermaria et al. (2015).[30] They reported that no significant changes were observed in the moisture content of the kefiran films when microorganisms were included. Our findings are in contrast with, Sánchez-González et al., (2014). They reported that the presence of bacteria in the film matrix increased the moisture content of the film.[46] Regardless of the nature of the films, the incorporation of lactobacilli into sodium caseinate, isolate pea protein, and methylcellulose films increased the moisture content of the edible films.[47] They explained that this phenomenon could be attributed to the higher water retention capacity of the microorganisms which ensures their survival. The higher moisture content in gelatin films is due to the water-holding capacities of gelatin that can retain water in the film matrix and it is mainly because of the tendency to form hydrogen bonds with water molecules.[48] However, the difference between the moisture content values may be due to the chemical structure of the components or the difference in the water solubility of the films.[37]

3.4.3. Water Solubility

Based on the data demonstrated in Table 1, the solubility percentage of the films, after a 24 h immersion in water, was from 22.38 to 43.52%. The analysis of the results indicated that, the probiotic loaded films were not affected by the water solubility of films. This result is in agreement with those reported by Kanmani and Lim, (2013), who realized that the solubility percentage of the control and bacterial pullulan/starch blended films are not significantly different.[44] In contrast, the addition of probiotics into the sodium alginate, sodium carboxymethylcellulose, and collagen matrix weakened the polysaccharide-protein interactions and increased the solubility of the films.[47] Increasing the solubility of the films is one of the major advantages of releasing probiotic strains and has a direct relationship with the moisture content which is dependent on the wettability and the free surface energy.[49] Higher molecular weights and polypeptides crosslinking in the sourdough and the wheat flour films leads to lower water solubility in comparison with the gelatin films. The films with lower water solubility are preferable for food packaging in terms of lower water activity and possible contamination in the presence of water.[50]

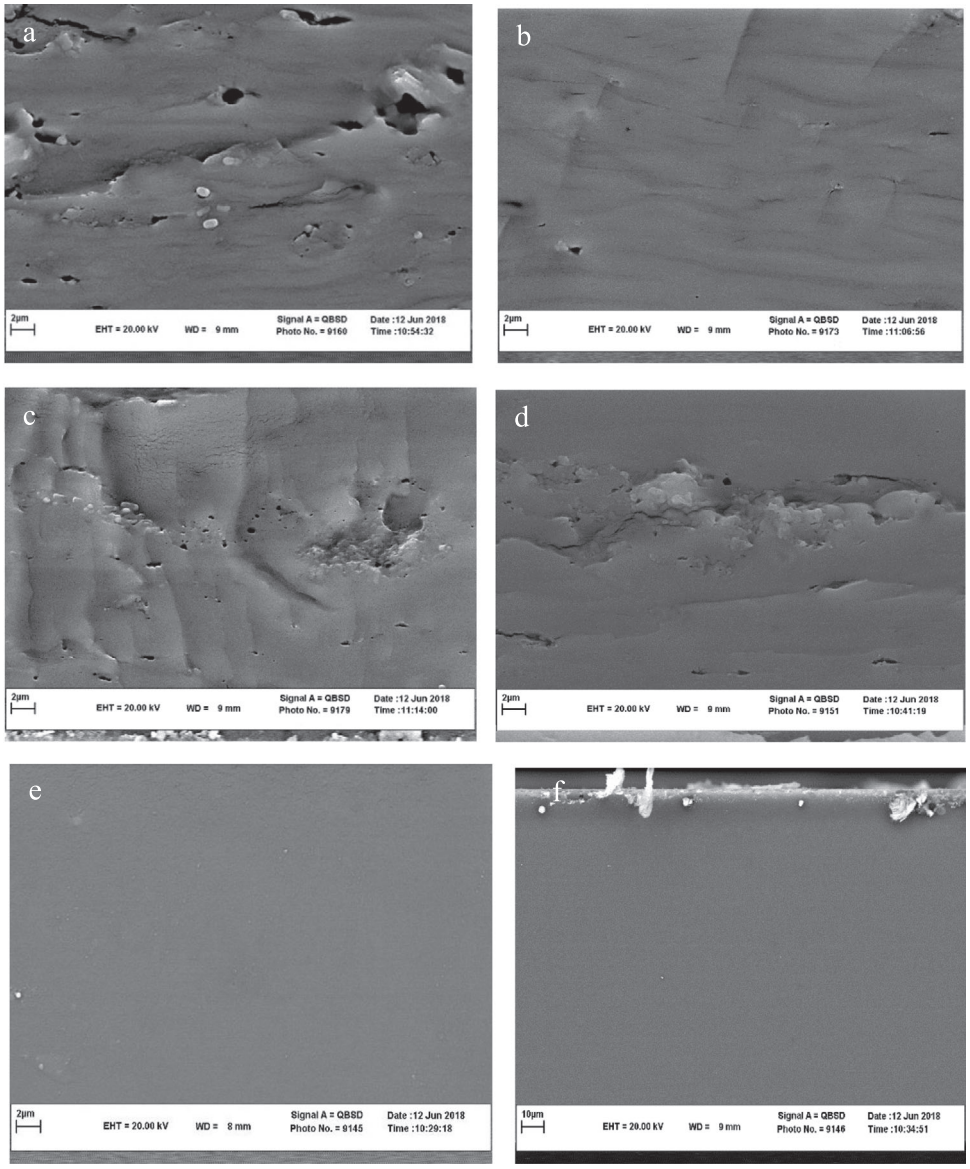


Figure 4. Scanning electron microscopy cross-section images of films, Films incorporating *L. plantarum* at sourdough film (a), wheat flour film (c) and gelatin film (e) (10^8 CFU gr^{-1}); films without incorporating *L. plantarum* at sourdough film (b), wheat flour film (d) and gelatin film (f).

3.4.4. Water Vapor Permeability (WVP)

As shown in Table 1, the probiotic sourdough film exhibited a higher WVP ($3.30 \pm 0.08 \times 10^{-7}$ g. $m^{-1}.h^{-1}.pa^{-1}$) than the other films. According to the results, the addition of the bacteria into the sourdough films, increased the WVP significantly ($p < 0.05$). This observed value for the probiotic sourdough film was about two times greater than that of the measured value for the control sourdough film. The wheat flour and the gelatin films were not affected by the addition of the probiotics into the films. As shown in **Figure 4**a,b, (SEM images of probiotic sourdough films) it can be seen that in the sourdough films, lactobacilli has changed the molecular structure of the film's matrix, destroyed the surface and enlarged the intermolecular space of the film surface.[46–47] Moreover, the probiotic cells might exist in the film's matrix as discontinuous particles, and thus inhibit the chain mobility of the polymers.[49] Therefore, the water vapor could penetrate more easily resulting in enhanced water vapor permeability. The WVP values were measured to find the water mass transfer through edible films. Lower WVP values are more desirable as they minimize unfavorable changes resulted by moisture in food products.[51]

3.4.5. Mechanical Properties

Table 1 shows tensile strength (TS) and elongation at break (%EAB) for the films used in this research. The wheat flour film exhibits a higher tensile strength (7.32 ± 0.5 Mpa) than the two other films. Incorporating the bacteria did change the TS of all the films except the gelatin films. On one hand, Ebrahimi et al. (2018) and Gialamas et al. (2010) reported that Lactobacilli cannot

affect the mechanical properties of sodium caseinate films.[7–52] On the other hand, it is in contrast with the results of Kanmani and Lim, 2013. They reported that the films' TS decrease when the amount of starch increases. The films that were made with pure starches exhibited the lowest tensile strength values.[44] As mentioned before, lactobacilli changed the molecular structure of the sourdough films' matrix and destroyed the surface of it and enlarged the intermolecular space of the film's surface, therefore, the TS significantly ($p < 0.05$) decreased in bacterial sourdough film.

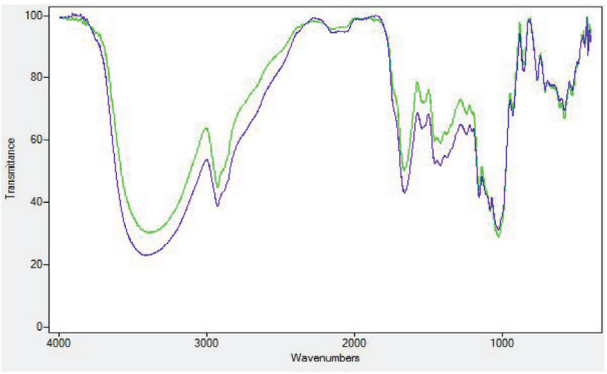
The higher elongation values belonged to the bacterial sourdough film (95.10 ± 10%). The values of EAB for control sourdough films were similar to those which were reported for the rice flour film (48.57%), and semolina flour films (58.78%).[53,54] The values of EAB in the sourdough and wheat flour films were increased with loading bacteria. The enhanced EAB value might be due to the change in the gluten structure caused by the bacteria's activity.[55] Increasing the chain organization can potentially optimize the molecular packing.[56] The EAB of the gelatin films was not affected by the presence of the bacteria in the films ($p < 0.05$). However, the wheat flour and the sourdough films showed a higher flexibility compared to the gelatin films, which can be due to the protein network structure. Therefore, the gelatin films indicated more desirable mechanical properties than sourdough and wheat flour films. The mechanical properties of the films are closely related to the distribution and the density of the intramolecular interactions and the polymer chains of the film matrix.[57] Probably, lactobacillus activities reduces the intermolecular interactions and the polymers chains, and thus leads to in an increase in the lengths of the films due to stretches. The films that have the gluten's mechanical properties and the water barrier properties are strongly affected by the presence of water or other plasticizers.[58]

3.4.6. Microstructural Properties

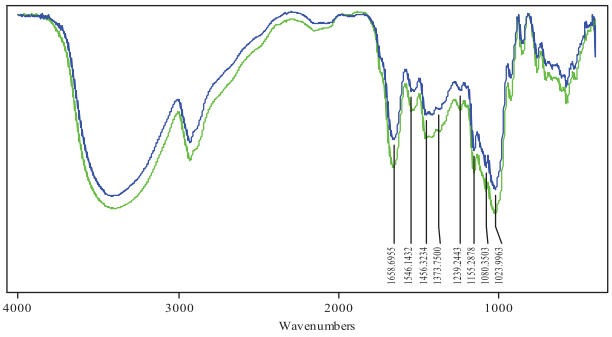
As shown in Figure 4, there was a significant structural difference between the sourdough, the wheat flour and the gelatin films. This can explain why these films act differently. The films that were fabricated with gelatin showed a more compact structure while a coarser and flaky structure was observed in the sourdough and the wheat flour films. The incorporation of probiotic cells did not cause a significant change in the structural conformation of the gelatin films. As it can be seen, the control sourdough and the wheat flour films (Figure 4b,d) were smooth with compact surface structures, whereas the probiotic sourdough and the wheat flour films (Figure 4a,c), had rough surfaces with some pores and cavities. These observations are in line with previous studies [6-7-30]. The proliferation of lactobacilli during the incubation of the films and the production of lactic acid could result in this structural change and affect the physical properties of the films.[44]

3.5. FTIR Analysis

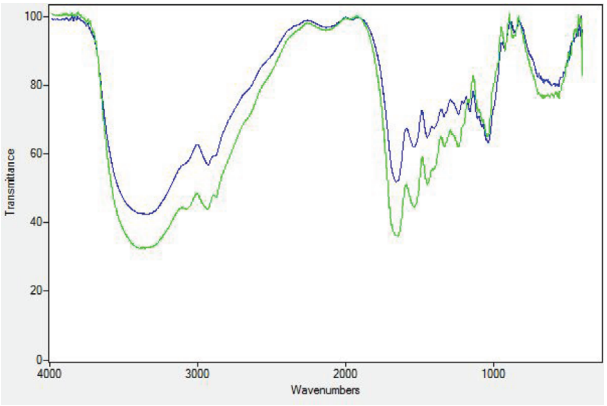
Figure 5 shows the FTIR spectrum of the films in the range of 400–4000 cm^{-1}. The spectral range was placed in the main transmittance peaks as follows: A peak at 3400 cm^{-1} was due to –OH



(A)



(B)



(C)

Figure 5. Fourier-transform infrared spectroscopy (FT-IR spectrum) of sourdough, wheat flour and gelatin films. (A). SDF = sourdough film, and PSDF = probiotic sourdough film (B). WF = wheat flour film, and PWF = probiotic wheat flour film (C). Gel = gelatin film, and PGF = probiotic gelatin film.

stretch due to the moisture. The spectra of the sourdough films obtained from show bands in the wavenumber of 2927 cm^{-1} were due to the C–H stretching vibrations in the films.[53] A series of bands were found in the region between 930 and 1155 cm^{-1}, which might be a response to C–O and C–C stretches. It also may be due to the deformational vibrations of CCH, COH and HCO bond The bands observed in the region of 1600–1700 cm^{-1}

(amide I) and 1500–1600 cm^{-1} (amide II), are closely related to C–O stretch and N–H bend.[56] The band situated at the wavenumber of 1081cm^{-1} corresponded to the glycerol (–OH group) which was added as a plasticizer.[59] The analysis of FTIR spectra indicated that the peaks of the control and the probiotic films were in the same regions. The incorporation of probiotics did not modify the FTIR spectra and this result was consistent with the research conducted by Pereira et al. (2017).[41] The presence of the bacteria could not significantly change the molecular structure of the films' samples. Therefore, it is concluded that no interactions occurred between the probiotics and the films' matrices.

4. Conclusions

In this study, sourdough, which is a unique biopolymer, was studied in order to develop novel edible films. The incorporation of probiotic cells into sourdough, wheat flour, and gelatin films can affect the physical and mechanical properties of the films. The sourdough films exhibited the best rank in protecting the *L. plantarum*, and the wheat flour films were the second. While gelatin films exhibited lower cell viability (than the other films) after 40 days of storage at 4 °C. Bacterial incorporation in the sourdough film significantly reduced WVP. It also weakened the mechanical properties of the film. The elongation at break significantly increased in the probiotic sourdough and wheat flour films. The thickness, the total soluble matter, and the moisture content of the control and the bacterial films were not different in all cases. In this study, the films were prepared in pure form, the mechanical and physicochemical properties of these films can be enhanced by using improving agents and other plasticizers. The main achievement of this study was the introduction of sourdough as an edible film that is suitable for the protection of probiotic bacteria, which in addition to its health-promoting properties, also has appropriate physicochemical properties.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

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

Keywords

edible films, *Lactobacillus plantarum*, simulated gastrointestinal conditions, viability

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