Improvement of set yogurt shelf life and quality by protective edible layers incorporated with Lactobacillus plantarum

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Abstract

In this study, layers were designed to protect yogurt using sourdough powder, wheat flour, and gelatin incorporated with *Lactobacillus plantarum*. The antimicrobial and antifungal (against *Aspergillus terreus*) characteristics of designed layers were investigated *in vitro*. After yogurt preparation, designed layers were placed on the surface of samples and stored at 4°C for 21 days. The physical and microbial properties and susceptibility to the growth of *A. terreus* in samples were assessed. For sensory evaluation, samples mixed with layers and yogurt without layers were evaluated. The *in vitro* antimicrobial and antifungal results indicated that the bacterial gelatin layer had the largest inhibitory zone, and the bacterial sourdough layer had the maximum inhibition growth against *A. trreus*. The viability of *L. plantarum* in the sourdough layer remained more than 10^6 CFU/g after two weeks on the yogurt. The designed layers could reduce the syneresis and pH of yogurt during storage in all cases. The sourdough and wheat flour layers could prevent fungal growth for at least 7 days. The taste of yogurt mixed with the bacterial wheat flour layer received the highest score, while the taste of the yogurt that had been protected with the sourdough layer was better than other samples.

1. Introduction

The progressive shift of consumers to health-oriented digestive products is driving the yogurt market. The global yogurt market is expected to record a compound annual growth rate of 4.5% during the period 2020–2025. Also, the pandemic of COVID-19 had a short-term positive effect on the retail yogurt market. The functional health benefits of yogurt caused in this period the market to have more demand as consumers were followed better diets and focused on their gut health (Mordorintelligence. Com, 2020).

As the yogurt market grows, the production of yogurt with high quality should be considered. In some traditional yogurts such as Greek-style yogurts that are made from whole milk, the milk does not become homogenized because the thin cream layer formation on the top of the product is desired (Chandan, 2017). Although the market demand for yogurt with a cream layer has increased (Das et al. 2019), because of the high cost of full-fat milk, the possibility of producing yogurt with a cream layer in the industry has been limited. Also according to market demand for traditional yogurt, there is the probability of fraud in this kind of product is also possible.

Generally, yogurt is not a very stable product, and its shelf-life is limited to about 3 weeks in cold storage and 2–3 days at room temperature (Lacroix & Lachance, 1990). Moreover, this product is particularly vulnerable to fungal growth due to its pH, water activity, and nutritional profile. Fungal contamination can cause discoloration, off-flavors, and alterations in texture and appearance, leading to loss of quality, waste, and consequently, economic losses. Fungi could tolerate acid, and easily contaminate all stages of yogurt processing (Delavenne et al., 2015).

Research indicated that the most important fungal spoilage generous of yogurt in Iran consists of *Aspergillus*, *Penicillium*, *Mucor*, and *Stemphylium*. *A. terreus* is also the most frequent species (45.35%) that caused fungal contamination in yogurt (Moshtaghi et al., 2015). *A. terreus* is the main fungus that can contaminate stored food products in tropical and subtropical climates, and it is thermophile and the minimum water activity for its growth is reported to be 0.78 at 37°C (Pitt, 2009).

A protective layer on the surface of yogurt with antimicrobial and nutritional properties could limit the penetration of oxygen and spoilage agents and improve the shelf life of the yogurt (Corsetti & Settanni., 2007; Delavenne et al., 2013). In this study, we were inspired by the creamy layer of the traditional yogurt and designed protective edible

layers with functional properties to improve the sensory and quality properties of yogurt. The protective layer was prepared based on wheat sourdough, wheat flour, and bovine bone gelatin incorporated with *L. plantarum*, and their antimicrobial and antifungal properties were assessed *in virto*. Also, the effect of designed layers on the shelf life, physicochemical, and sensory properties of yogurt samples was evaluated.

2. Material And Methods

2.2. *Preparation of layers*

The wheat sourdough powder was prepared by the method of Kalantarmahdavi et al. (2021). The whole wheat flour and bovine bone gelatin were purchased. The layers were prepared by adding 10 g of dry matter to 100 ml of boiling water. The glycerol as plasticizer at the ratio of 10% (w/w, dry matter) was added. After cooling of suspensions to 40°C, *L. plantarum* suspension (10^8 CFU/ml) was added and homogenized. The layers formation was done by casting method into plastic Petri dishes with 8 cm diameter. Then drying was taken place at 37°C for 24 h. The layers without bacteria were also prepared by the mentioned method.

2.3.3. *In Vitro antimicrobial activity of layers*

Stock cultures of *Bacillus cereus* (PTCC 1154), *Staphylococcus aureus* (PTCC 1431), *Escherichia coli* (PTCC 1396), *Listeria monocytogenes* (PTCC 1298), *Salmonella typhimurium*, and *Pseudomonas aeruginosa* were kept frozen in Brain Heart Infusion Agar (BHI, Merck KGA, Germany) supplemented with 30% glycerol. For activating the cultures, they were transferred into BHI broth and incubated at 37ºC for 24h. The overnight cultures were used to take an aliquot for transferring into BHI, and resulted in suspensions were incubated at 37ºC. The antimicrobial activity of the prepared layers was assessed through the Agar disk diffusion assay (López-Malo et al., 2020). Target strains were inoculated (10^6 CFU/cm^2) in Mueller Hinton agar (Merck KGA, Germany). Three disks (6 mm in diameter) of prepared bacteria layers and three disks (6 mm in diameter) of layers without bacteria were placed on the surface of the cultured plates and were incubated at 37 ºC for 24 h. Inhibition zones were measured with a manual caliper.

2.3.4. *In Vitro antifungal activity of layers*

Lyophilized culture of *A. terreus* PTCC 5283, isolated from soil, was purchased from the Iranian Research and Organization for Science and Technology. Lyophilized fungi were activated in aseptic conditions. Afterward, the fungi colonies were transferred into the test tubes with inclined Potato Dextrose Agar (PDA) (Merck KGA, Germany), and incubated until when the mycelium grew. The spores were collected by washing with distilled water containing 0.05% (w/v) tween 80. The fungal suspension was prepared by reference method for broth dilution antifungal susceptibility testing of filamentous fungi (Wayne, 2008). The spore's concentration was determined using UV–vis. spectrophotometer (Mecasys Co., Korea) at 530 nm and within the optical absorption range (80–82%). The suspension contained 10^6 spores per ml. Spore dilution set to 10^3 via serial dilution. The antifungal activity of the layers was evaluated according to Sánchez-González et al (2013) with slight modifications. The spore's suspension (10^3 spores/ml) was inoculated onto the surface of the plates then the layers were cut tailor-made to plates and were placed on the surface of them. The plates were incubated at 25°C for one week. After the incubation period, the growth and non-growth of colonies were investigated.

2.4. *Preparation of yogurt samples*

Yogurt samples were prepared by heating whole milk (3.9% of fat content) at 92°C for 12min, cooling it to 44°C, and inoculating with *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (YZ-1, micromilk S.R.I., Italia; 2 kg of batch starter/100 kg milk) as a starter culture. Then the inoculated 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 surfaces were then covered with circular prepared layers and refrigerated overnight at 4°C.

2.5. *Yogurt characterizations*

2.5.1. *pH value*

The pH values of the yogurt samples were measured using the pH meter (Martini, Mi 151, china) after calibrating with fresh pH 4.0 and 7.0 standard buffers.

2.5.2. *Syneresis*

Syneresis was determined using centrifugal methods (Domagała et al., 2012). 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 %.

2.5.3. *Enumeration of L. plantarum and yogurt starter*

Yogurt samples with top layers were kept for 21 days at 4°C to evaluate the viability of *L. plantarum* entrapped in protective layers and the interaction between the protective layer and yogurt starter bacteria. For every test, 1 g of the top layer and 1 g of the yogurt sample were homogenized with sterile peptone water (1 g/L) separately. The appropriate dilutions were plated on set De Man, Rogosa, and Sharpe agar (MRS) (Merck KGA, Germany). Then, they were incubated in anaerobic conditions at 37°C for 48 h. The set MRS containing 10 mg/L of vancomycin for inhibition of yogurt starter growth was used for layers that were removed from the yogurt surface and set MRS for yogurt samples. 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 was performed using the colony count technique.

log CFU/g, CFU/g = CFU/plate × dilution factor.

2.5.4. *Antifungal characteristic of layers on yogurt*

The fungal spore suspension was prepared as the mentioned method. The antifungal activity of the layers on the yogurt surface was tested according to Gialamas et al (2010) method with slight modification. Briefly, after yogurt preparation, 0.1 mL of *A. terreus* spore suspension (10^3 spores/ml) was inoculated on the surface of the yogurt samples. The layers were cut tailor-made to containers and placed on the inoculated surface of yogurt samples. The fungal colonies were detected after 14 days of incubation at 25 ºC.

2.5.5. *Sensory analysis*

After the preparation of yogurt, samples were covered with the layers and evaluated for 20 days at 4°C. Seven educated panelists were chosen for sensory evaluation using qualitative descriptive analysis. The samples were divided into two parts, in Part I: the layer with the yogurt sample was mixed at the time of consumption, and in part II: The layer was removed from each sample, and yogurt was assessed without the layer. All samples were coded by 3 random numbers. The main attributes of taste, texture, and overall acceptability were used to evaluate. A 5-cm unstructured hedonic scale was used with extremes of “(1) extremely disliked” and “(5) extremely liked” (Stone & Sidel, 2004). Sensory evaluation was done in 5, 10, 15, and 20 days.

2.6. *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 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. *Antimicrobial activity of layers*

The inhibitory zone diameters (mm) of designed layers are presented in Table 1. The bacteria-less layers did not exhibit antimicrobial activity in all cases as expected. The mean diameter of inhibition zones of gelatin discs containing *L. plantarum* was 2.34 ± 11.83 mm. The sourdough and wheat flour discs incorporated with *L. plantarum* had no inhibition zone; nevertheless, colonies of tested bacteria did not cover the discs. This inhibition zone would be great and represents that a high amount of bacteria have been inhibited.

Table 1
Antimicrobial activities of tested layers (With and without *Lactobacillus plantarum* PTCC 1745) against target microorganisms.

inhibition zone (mm)						
Protective layer	*Escherichia coli PTCC 1396*	*Staphylococcus aureus PTCC 1431*	*Bacillus cereus PTCC 1154*	*Listeria monocytogenesis* PTCC 1298	*Salmonella typhimurium*	*Pseudomonas aeruginosa*
SD	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$
WF	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$
Gel	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$	$0.00 \pm 0.00^{a,C}$
SD.pro	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$	$600 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$
WF.pro	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$	$6.00 \pm 0.00^{a,B}$
Gel.pro	$10 \pm 0.80^{c,A}$	$12.50 \pm 0.57^{b,A}$	$8.00 \pm 1.15^{c,A}$	$14.5 \pm 0.57^{a,A}$	$14 \pm 0.89^{a,A}$	$12 \pm 0.50^{b,A}$

Data reported are mean values ± standard deviations. Different letters are significantly different at $P < 0.05$. SD = PDA plates with sourdough layer, WF = PDA plates with wheat flour layer, Gel = PDA plates with gelatin layer, SD.pro = PDA plates with probiotic sourdough layer, WF.pro = PDA plates with probiotic wheat flour layer, Gel.pro = PDA plates with probiotic gelatin layer.

Two bacteriocins isolated from wheat sourdough, namely bacteriocin bavaricin (produced by *L. sakei* strain) and bacteriocin plantaricin (produced by *L. plantarum*) have been reported to show antimicrobial activity (Arena et al.,

2020). Similar inhibition zone of sourdough and wheat flour layers with *L. plantarum*, could be attributed to the inability of the layer matrixes to release antimicrobial compounds. In this line, research proved that coatings, containing *L. plantarum*, had significant antimicrobial effects due to the better release of antimicrobial metabolites (Marín et al., 2019; Trabelsi et al., 2014). These inhibition zones could be considered small; although, they suppressed high counts of bacteria. Even more, it is proposed that these layers could be utilized as coatings or wrappings thus layers will be in contact with the food surfaces, and observed antimicrobial activity could be quite enough. Not only that, well-manufactured food products will have low bacterial counts, that's why observed inhibition would be more than adequate. Furthermore, gelatin layer, containing *L. plantarum*, had acceptable antimicrobial diffusion compared to the two other layers, which may be due to the hydrophilic nature of the gelatin protein that causes the better release of antimicrobial compounds (Theerawitayaart et al., 2019).

3.3. *In Vitro antifungal activity of layers*

Antifungal activity of the layers indicated positive effects on growth of *A. terreus* compared to the control (Fig. 1g). As shows in Fig. 1 (a, and b), Sourdough layers (with and without bacteria) had the most antifungal effects. According to previous studies, sourdough has an extensive antifungal activity due to the wide range of metabolites (Fekri et al., 2020; Arena et al., 2020). In a related study, two strains of *Lactobacillus paralimentarius* and *Lactobacillus rossiae*, isolated from wheat sourdoughs, were introduced for their significant antifungal activity against three indicator cultures, i.e., *Eurotium repens*, *Aspergillus japonicus*, and *Penicillium roseopurpureum* (Garofalo et al., 2012). The main antifungals of sourdoughs, namely 2-hydroxyisocaproic acid, and 3-phenyllactic acid are originated from the LAB amino acid metabolism (Hassan & Bullerman, 2008). As former studies reported, 3-phenyllactic acid could be produced by *L. amylovorus*, *L. reuteri*, and also some strains of *L. brevis* (Ryan et al., 2011; Gerez et al., 2009). Phenolic acids are another group of antifungal metabolites in sourdough, which include derivatives of cinnamic acid and benzoic acid. Free phenolics production is catalyzed by LAB through cereal enzymes or chemical reactions, during sourdough fermentation (Gänzle, 2014).

Wheat flour and gelatin layers also, showed antifungal property (Fig. 1). According to the literatures, coatings and films are commonly intended to protect the product against attack by fungi (Embuscado & Huber 2009). It seems that the oxygen barrier capacity of films leads to an inhibitory effect against fungi. In this regard, Muangrat and Nuankham (2018) reported that covering the strawberries with rice flour film caused to increase in the storage time to 9 days, with no yeast and fungi growth. The obtained data in the present study indicated that both control and bacterial layers were able to inhibit the growth of the fungus.

3.4. *Yogurt characterizations*

3.4.1. *pH value*

As shown in Fig. 2 (A), the pH in the control sample decreased significantly ($p \leq 0.05$) from 4.2 to 4 during the storage period. Whereas the pH had no significant changes for 14 days in all treatments. The pH of yogurt with bacterial gelatin layer and bacterial wheat flour layer were significantly decreased in week 3. The reduction of pH in the sample with the wheat flour layer containing bacteria was significantly lower than in other treatments at the end of the storage time (4 to 3.86). The pH changes of samples with sourdough layers (with and without bacteria) and gelatin layers (without bacteria) were constant until the end of storage time. This phenomenon indicates that the post-acidification capability of these layers was weak. These results were in line with previous results of Ortakci and Sert (2012) they reported that free and encapsulated Lactobacillus acidophilus in yogurts followed a similar pH rate during storage at 4°C for 4 weeks (Ortakci & Sert, 2012). The low permeability of layers to oxygen

improves microaerophilic growth of starter bacteria resulting in more acid production (Kasapis, 2020). The layers matrix appears to inhibit the release of acid produced by the bacteria entrapped in it. The entrapping of probiotic bacteria in the layer matrix can solve the problem of increasing the acid and the disorders to organoleptic changes in the yogurt.

3.4.2. *Syneresis*

The release of whey is a common problem in yogurt, and its high level indicates the low quality of the product. It is defined as the expulsion of whey from the casein network (Allgeyer & Lee 2010). Common causes of syneresis include high incubation time, disproportionate whey protein to casein ratio, low solid content, and physical mishandling of the product during storage and distribution (Mwizerwa et al., 2017). Syneresis reduction can be observed in yogurt samples with top layers, as illustrated in Fig. 2 (B). The percentage of syneresis in the control sample was increased due to the activity of starters during the storage period, while the presence of a layers on the surface of yogurt caused a downward rate in syneresis of samples. According to previous research, the level of syneresis in probiotic yogurt increased during the storage period at 4°C (Mortazavian et al., 2006). Generally, the shrinkage of the three dimensional protein network and gel stimulates syneresis (Shan et al., 2013). It seems that the presence of layers on the surface of yogurt can absorb whey isolated from the yogurt gel. In fact, the presence of layers can help physically reduce syneresis. Therefore, the higher the water holding capacity or porosity of the layer structure, the more water can absorb from the yogurt gel. It seems that the amount of water absorption in the gelatin layers (with and without bacteria) are higher than the others. Water-holding capacity in gelatin mainly depends on the content of hydrophilic amino acids and hydroxyproline (Ninan & Abubacker, 2011). In all samples the presence of bacteria in layers had no significant effect on the syneresis.

3.4.3. *Enumeration of L. plantarum and yogurt starters*

The changes in the counts of *L. plantarum* entrapped in layers and free in yogurt during storage are shown in Fig. 3 (A). The *L. plantarum* number decreased 5.15 log from the initial number (8.70 Log) in control (yogurt containing free cells). This reduction is probably due to the bactericidal activity of lactic acid and acetic acid produced by the bacteria and β-galactosidase activity (post-acidification) (Ortakci & Sert, 2012). The viable cell of *L. plantarum* entrapped in layers of sourdough, wheat flour, and gelatin on yogurt samples was reduced by 2 logs, 2 log, and 2.36 log, respectively. As can be seen, the bacteria in the sourdough layer were able to remain on the threshold for probiotics (10^6 CFU/g) for up to two weeks. Bandiera et al. (2013), explain that decrease in the populations of probiotic bacteria probably due to stress caused by the low temperature. Another hypothesis describes that osmotic shock during layers rehydration because of the high moisture content of yogurt, is the reason for decreasing the number of *L. plantarum* (Meg et al, 2008). Because of the nutritional environment of yogurt with the appropriate pH for probiotics survival, the bacteria were able to recover, which led to stability in the number of bacteria entrapped in layers until the end of storage. Ziare et al. (2012) indicated that microencapsulation in calcium alginate-resistant starch mixed gel was found to improve the viability and maintain a suitable post-acidifying activity of probiotics in yogurt after 1 month of storage at 4ºC.

Figure 3 (B), shows that there was a constant decline in the population of yogurt starters in all samples during the storage. All samples maintained a high level of starter bacteria during the 3-week cold storage. The highest number of starter bacteria was for yogurt with the sourdough layer containing *L. plantarum* (6.9 log) and for yogurt with the non-bacterial sourdough layer (6.65 log) on day 21. Yogurt with the non-bacterial gelatin layer and control had the lowest number of starter bacteria after storage time. The low permeability of the protective layer to oxygen decreases the growth of undesirable microorganisms and improves the microaerophilic growth of starter

bacteria (Kasapis, 2020). 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 viability of the starter culture of this product (Delavenne et al., 2013). In this study, the layers could be the protective layer on the surface of yogurt to limit oxygen and spoilage agents and also can maintain the viability of starter cultures.

3.4.4. *Antifungal characteristics of layers in yogurt*

The effect of the layers on fungal contamination of yogurt during storage at 25°C is shown in Fig. 4. A complete lack of growth of *A. terreus* was observed only in yogurt covered with a sourdough layer (bacterial and non-bacterial) after 14 days (Fig. 4, (a)). The surface of the control sample (without layer) was covered with fungus after 14 days of incubation (Fig. 4, (g)). In all samples that were covered with bacterial and non-bacterial layers, fungal contamination was observed during storage, nevertheless, compared to the control sample, a significant delay in the fungal growth process was observed.

Our results are relevant to Guiamars et al. (2020) they studied the effect of films and coatings based on whey protein containing *Lactobacillus buchneri* for the control of *Penicillium nordicum* in cheese. The films and coatings containing *L. buchneri* cells prevented fungal contamination for at least 30 days, while control cheeses with films and coatings either without bacteria showed fungal contamination during that period. As shown in Fig. 4 (a, and b), sourdough layers (with and without bacteria) have a greater effect on inhibiting fungal growth than wheat flour and gelatin layers. This results was related to the results of antifungal activity *in vitro* condition. The antifungal properties of sourdough have already been discussed.

3.4.5. *Sensory Analysis*

Tables 2 and 3 shows the effect of bacterial and non-bacterial layers on yogurts in 21 days of storage. As previously mentioned, the samples were divided into two categories: category I is yogurt samples mixed with layers (Table 2), and category II is yogurt samples without layer (Table 3). Between the yogurt samples mixed with the top layers, yogurt samples mixed with the non-bacterial sourdough layer, and the non-bacterial wheat flour layer, obtained the highest score in taste, texture, and overall acceptance. The lowest score belonged to the control samples, and the yogurt mixed with the bacterial sourdough layer. Between the yogurt samples without the top layers, yogurt samples with the non-bacterial wheat flour layer obtained the highest score in taste, texture, and overall acceptance. The lowest score belonged to the yogurt with the bacterial sourdough layer. In the case of yogurt mixed with bacterial sourdough layer, the high increase in sour taste probably reduced the score. It seems that sourdough has a sour flavor by nature, and *L. plantarum* activities have doubled the sour flavor when mixed with yogurt causing the undesirable sour flavor and texture.

Table 2
Sensory analysis of designed layers (With and without *Lactobacillus plantarum* PTCC 1745) mix with yogurt.

	treatments						
attributes	Yog + Gel.pro	Yog + Gel	Yog + SD.pro	Yog + SD	Yog + WF.Pro	Yog + WF	C
day 1							
Flavor	4.45 ± 0.21^{b}	4.42 ± 0.17^{b}	4.42 ± 0.17^{b}	4.85 ± 0.17^{a}	4.43 ± 0.11^{b}	4.85 ± 0.14^{a}	4.28 ± 0.19^{b}
Texture	4.71 ± 0.11^{a}	4.71 ± 0.15^{a}	4.42 ± 0.13^{b}	4.71 ± 0.14^{a}	4.14 ± 0.16^{b}	4.71 ± 0.16^{a}	4.14 ± 0.16^{b}
Acceptability	4.42 ± 0.11^{b}	4.50 ± 0.17^{b}	4.57 ± 0.13^{b}	4.71 ± 0.19^{a}	4.42 ± 0.16^{b}	4.71 ± 0.12^{a}	4.28 ± 0.16^{b}
day 21							
Flavor	4.00 ± 0.10^{a}	3.57 ± 0.11^{b}	3.00 ± 0.18^{c}	3.85 ± 0.15^{a}	3.38 ± 0.16^{b}	3.71 ± 0.18^{b}	3.14 ± 0.16^{c}
Texture	4.14 ± 0.16^{a}	3.42 ± 0.13^{b}	2.85 ± 0.16^{d}	4.14 ± 0.19^{a}	3.42 ± 0.11^{b}	3.71 ± 0.18^{b}	3.14 ± 0.14^{c}
Acceptability	3.85 ± 0.16^{a}	3.44 ± 0.11^{b}	3.13 ± 0.19^{c}	4.14 ± 0.19^{a}	3.57 ± 0.16^{b}	3.71 ± 0.18^{a}	3.12 ± 0.17^{c}

Data reported are mean values ± standard deviations. Different letters are significantly different at $P < 0.05$. SD = sourdough layer, WF = wheat flour layer, Gel = gelatin layer, SD.pro = probiotic sourdough layer, WF.pro = probiotic wheat flour layer, Gel.pro = probiotic gelatin layer, C = Control (plain yogurt).

Table 3
Sensory analysis of yogurt protected with designed layers (With and without *Lactobacillus plantarum* PTCC 1745).

	treatments						
attributes	Yog + Gel.pro	Yog + Gel.C	Yog + SD.pro	Yog + SD.C	Yog + F.Pro	Yog + FC	C
day 1							
Flavor	4.55 ± 0.21^{b}	4.82 ± 0.16^{a}	5.00 ± 0.17^{a}	5.00 ± 0.18^{a}	4.85 ± 0.16^{a}	5.00 ± 0.16^{a}	5.00 ± 0.16^{a}
Texture	4.41 ± 0.10^{b}	4.14 ± 0.17^{b}	4.72 ± 0.10^{a}	4.14 ± 0.15^{b}	4.14 ± 0.11^{b}	4.14 ± 0.12^{b}	4.14 ± 0.19^{b}
Acceptability	4.21 ± 0.15^{b}	4.14 ± 0.17^{b}	4.00 ± 0.16^{b}	5.00 ± 0.14^{a}	4.42 ± 0.15^{b}	4.18 ± 0.18^{b}	5.00 ± 0.16^{a}
day 21							
Flavor	3.14 ± 0.15^{c}	3.28 ± 0.11^{c}	2.71 ± 0.18^{d}	2.57 ± 0.19^{d}	3.42 ± 0.16^{b}	3.71 ± 0.48^{a}	3.28 ± 0.16^{c}
Texture	3.42 ± 0.16^{b}	3.14 ± 0.13^{c}	2.85 ± 0.16^{d}	2.57 ± 0.19^{d}	3.42 ± 0.11^{b}	3.71 ± 0.48^{a}	3.14 ± 0.14^{c}
Acceptability	3.14 ± 0.16^{c}	3.14 ± 0.11^{c}	2.71 ± 0.19^{d}	2.57 ± 0.19^{d}	3.42 ± 0.16^{b}	3.71 ± 0.48^{a}	3.14 ± 0.17^{c}

Data reported are mean values ± standard deviations. Different letters are significantly different at $P < 0.05$. SD = sourdough layer, WF = wheat flour layer, Gel = gelatin layer, SD.pro = probiotic sourdough layer, WF.pro = probiotic wheat flour layer, Gel.pro = probiotic gelatin layer, C = Control (plain yogurt).

The formation of flavor in yogurt is the result of a complex network of processes in which the end product results in the development of aroma and flavor compounds. Glycolysis, lipolysis, and proteolysis are the three main processes that contribute to flavor development (Das et al., 2019). Yogurt's unique flavor comes from the lactic acid produced by the starter, several aromas compounds naturally present in the milk, and those produced by the fermentation process (Ott, et al., 1997; Tamin & Deeth, 1980). Acetaldehyde, ethanol, acetone, diacetyl, and 2-butanone play an important role in the desirable flavor compounds found within yogurt (Tamin & Robinson, 1999). The predominant flavor of yogurt is the sourness produced by the starter cultures. Lactic acid is slightly volatile, so it is not usually related to the aroma profile of yogurt, but it does play an important role in the overall flavor profile of yogurt. Most yogurts contain approximately 0.8–1.0% lactic acid with a pH below 4.6. When the pH drops below 5, consumers can detect yogurt sourness in the absence of sweeteners and other flavorings. Some organic acids, such as acetic and formic acid may be produced by the starters at lower concentrations and only contribute slightly to the flavor of the finished product (Hutkins, 2008). Acetaldehyde is a two-carbon aldehyde found within yogurt between 8–40 ppm and is the most important compound in yogurt flavor (Kneifel et al., 1992). Diacetyl does play an important role in the delicate full flavor of yogurt when acetaldehyde is present at lower concentrations. The typical concentration of diacetyl in yogurt ranges from 0.2–3.0 ppm (Pourahmad & Assadi, 2005). It seems that the yogurt sample with non-bacterial wheat flour layer in both cases mixed with yogurt and without mixing with yogurt preserves the flavor and quality of yogurt samples in storage time.

4. Conclusions

Due to the elimination of the homogenization process in traditional yogurts, as might be expected, the fat globules are clustered and a creamy surface is formed, which prevents penetration of fungi and microbes in yogurt. On the other hand, this creamy layer has created challenges, including cheating due to the high cost of milk fat. In this study, simulating this creamy layer based on sourdough, wheat flour, and gelatin, incorporated with *L. plantarum*, are appropriate alternatives to develop natural antimicrobial and antifungal layers for improving the yogurt quality and shelf life. The results of this study showed that the bacterial layers presented antimicrobial and antifungal activity against target bacteria and fungi in vitro and in yogurt. Therefore it could have potential food applications. Generally, the inhibition properties of these layers make them adequate to use as wrappings or coatings of food products. The sourdough layers (bacterial and non-bacterial) inducted better antifungal properties than the other studied layers in the laboratory medium and in food model, due to the sourdough's antifungal nature. *L plantarum* could survive in the sourdough layer for more than 10^6 CFU/g. In this study, the sourdough and wheat flour layers had favorable effects on the physical, microbial, and sensory properties of yogurt samples. Therefore, based on the results of this research, sourdough and wheat flour films can be an optimizing candidate to enter the food industry as a bioactive edible film. However, complementary research is needed to evaluate its application in other food products.

Declarations

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Compliance with Ethical Standards

Conflict of interest: The authors declare no conflict of interests.

Author Contributions

Mahboubeh Kalantarmahdavi: Data curation, Investigation, Methodology, Writing-original draft, Software.

Amir Salari: Conceptualization, Methodology, Project administration, Data curation, Formal analysis, Validation Resources, Visualization, Roles, Writing – review & editing.

Saeid Khanzadi: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Data Availability

Raw data were generated at Ferdowsi University of Mashhad. Derived data supporting the findings of this study are available from the corresponding author (Saeid Khanzadi) on request.

Funding Declaration

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Figures

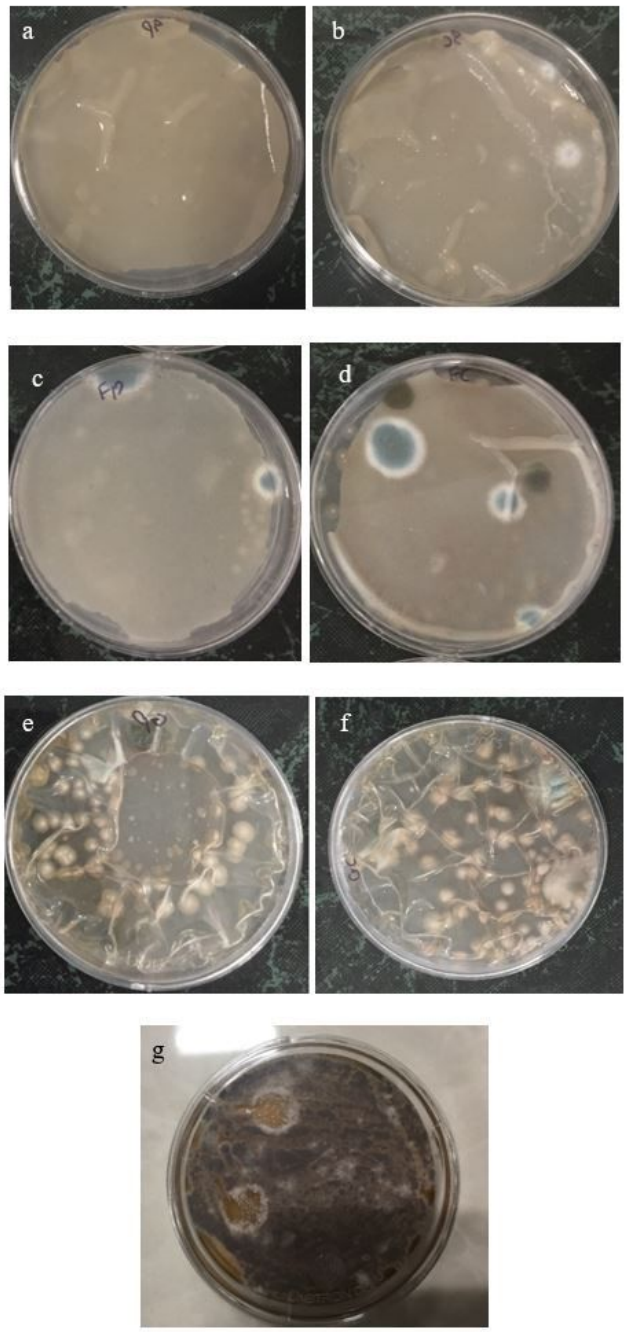
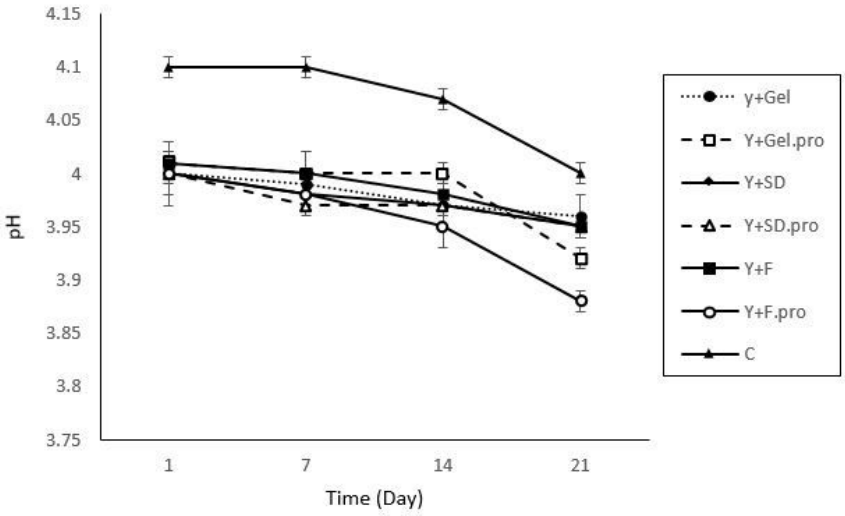


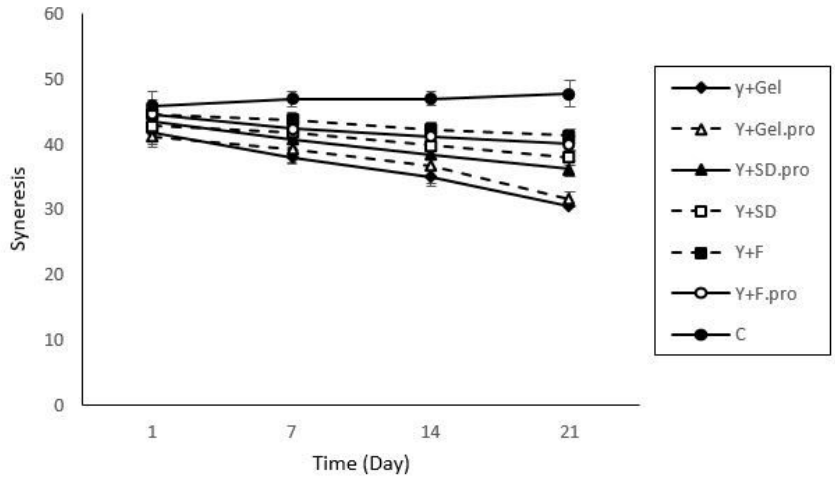
Fig. 1

Figure 1

Antifungal activity of protective layer against *A. terreus* by inoculation of a spore solution (10^3 spore/ml) on PDA. layers incorporating *L. plantarum* at sourdough layer (a), wheat flour layer (c) and gelatin layer (10^8 log CFU/gr) (e); layers without incorporating *L. plantarum* at sourdough layer (b), wheat flour layer (d) and gelatin layer (f); sample without layer- *A. terreus* control (g).



(A)

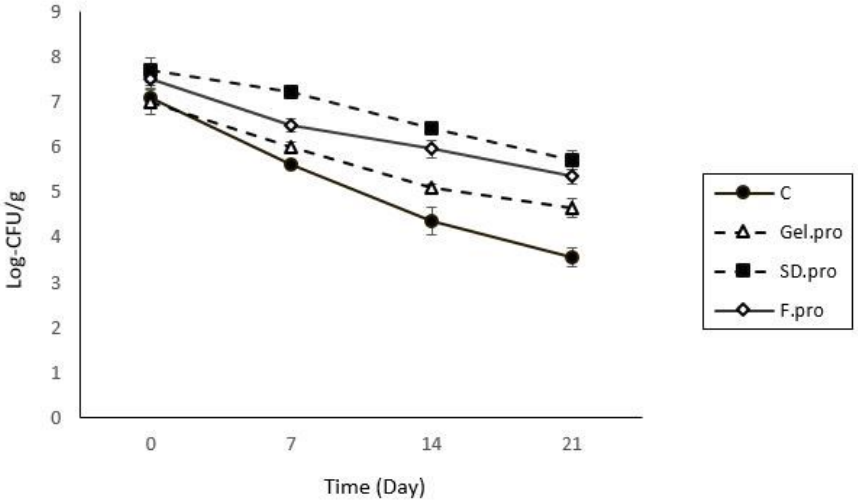


(B)

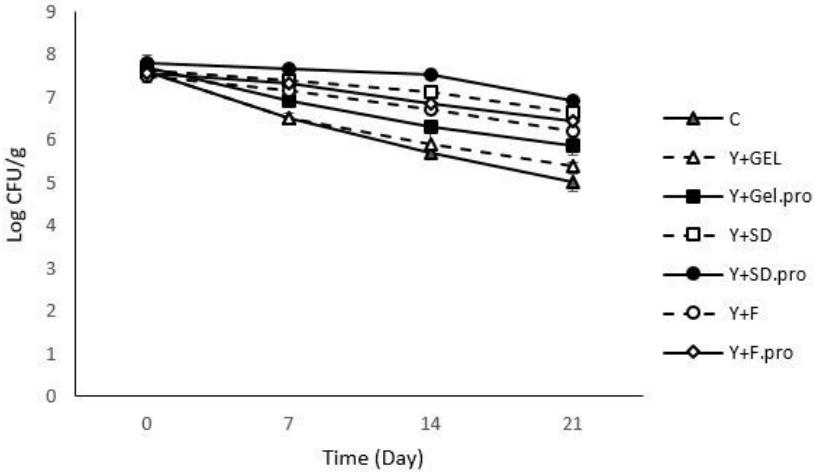
Fig. 2

Figure 2

(A), Effect of layers on the pH value of yogurt samples covered with layers and control, during storage at refrigerated (4°C). (B), Syneresis of treated yogurt samples with layers and control, during storage at refrigerated (4°C).



(A)



(B)

Fig. 3

Figure 3

(A), Viability of *L. plantarum* entrapped in layers in food model (yogurt) condition, during storage (21 days at 4°C). (B), Viability of yogurt starter with protective layers during storage (21 days at 4°C).

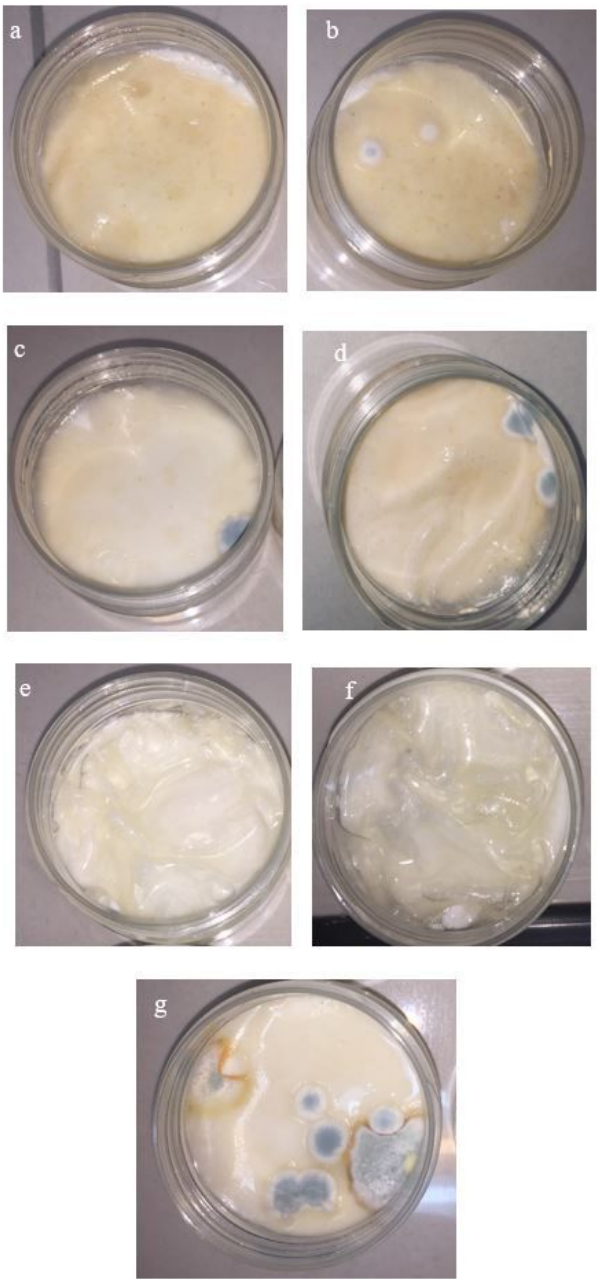


Fig. 4

Figure 4

Growth of *A. terreus* on yogurt samples after 14 days; yogurt covered with bacterial sourdough layer (a); yogurt covered with bacterial wheat flour layer (c) and yogurt covered with bacterial gelatin layer (e); yogurt covered with sourdough layer without *L. plantarum* (d); yogurt covered with wheat flour layer without *L. plantarum* (d) and yogurt covered with gelatin layer without *L. plantarum* (f); control (g).