



# Stability of *Bacillus coagulans* IBRC-M 10807 and *Lactobacillus plantarum* PTCC 1058 in Milk Proteins Concentrate (MPC)-Based Edible Film

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## Abstract

Controlled release of probiotic bacteria via edible films as a vehicle is a novel approach. Therefore, in this study, the stability of two probiotic microorganisms, *Bacillus coagulans* IBRC-M 10807 and *Lactobacillus plantarum* PTCC 1058, were investigated in milk protein concentrate (MPC)-based edible films under different temperatures. For this purpose, effect of four factors, the probiotics (*B. coagulans* IBRC-M 10807 and *L. plantarum* PTCC 1058), glycerol concentration (6, 7.5, and 9% w/w), storage temperature (−16, 4.5, and 25 °C) and storage time (3, 5, and 7 days) was investigated in edible films prepared using 10% w/w of milk protein concentrate containing 7 Log<sub>10</sub> CFU/g of probiotics. Then the viability of each probiotic bacterium as well as important characteristics of probiotic edible films: pH, moisture content, solubility, water vapor permeability, and whiteness index were evaluated for optimization. Probiotic type and glycerol concentration had a significant effect on live-cell count ( $p < 0.05$ ). The survival of *B. coagulans* spores was higher than that of *L. plantarum*. The optimum conditions included 9% glycerol concentration, −16 °C and 7 day storage, in which survivability of *B. coagulans* and *L. plantarum* was 6.67 and 6.53 Log<sub>10</sub> CFU/g, respectively. The overall results showed that MPC-based edible film could be a suitable carrier for probiotic bacteria to pack food stored at freezing temperatures.

**Keywords** Edible film · Milk proteins concentrate · Probiotics · Viability

## Introduction

Edible films and coatings are manufactured from biopolymers and food-grade additives. Biopolymers are defined as a continuous matrix of proteins, polysaccharides, or fats that are capable of altering food surface properties [10]. Food coatings have been used for many years to better preserve food products and increase their appeal. In addition to preventing drying and creating an attractive appearance, these coatings reduce the exchange of respiratory gases and the growth of molds and insects on foods or reduce chemical

and biochemical activity in them. In recent decades there has been a growing interest in the development of new technologies in the production and application of biodegradable films. They are being used as carriers of antioxidant, antimicrobial, enhancers, flavors, and colorings, as well as for enhancing nutritional value, enriching foods with vitamins and minerals [23].

Over the last decade, consumer demand for food products with health effects has increased [7]. Therefore, the role of food in the prevention of chronic diseases has received much attention. As a result, both producers and consumers show great interest in "functional foods". Foods containing probiotic bacteria have gained a significant attend of customers which led to an increase in their annual market [3, 4]. Probiotics are beneficial living bacteria that, if taken in sufficient counts, have health effects for the consumer [5, 6]. *Lactobacillus* strains are Gram-positive, micro-aerophilic, acid-resistant, non-spore-forming and low G + C group bacteria. These bacteria have a wide range of applications in the food industry as a starter and protective culture [8, 29]. *Lactobacillus* produces organic acids such as acetic acid, benzoic

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acid, and lactic acid as the main metabolites [28]. The antibacterial, antifungal and probiotic properties of LAB have been extensively studied [27]. *Lactobacillus plantarum* grows under low buffering capacity in the stomach and other bile salt secretions in humans and other mammals. In addition to applications in the food industry, and it is widely used in the pharmaceutical industry and significantly assists human medicine without any adverse effects. Recently, *L. plantarum* has been used in medical fields for the treatment of various chronic and cardiovascular diseases such as Alzheimer, Parkinson, diabetes, obesity, cancer, hypertension, urinary complications, liver disorders, etc. [15]. In the last 2 decades, attention has been given to *Bacillus* species as a probiotic due to adaptation to adverse conditions. *Lactobacillus sporogenes* was isolated and described in 1932 by Wlassow Horowitz and Nowotelnow and subsequently classified as *Bacillus sporogenes* but was never accepted as *Lactobacillus* because of its sporadic origin. Recently, evidence suggests that the bacterium has the same traits as *Bacillus coagulans* and was, therefore, transferred to that group. In general, *B. coagulans* is a bacterial species that forms lactic acid but of *Bacillus* genus [13].

One of the most important challenges in the production and processing of probiotic products is the low viability of probiotic bacteria due to susceptibility to harsh conditions within the food product as well as adverse gastrointestinal conditions. Microencapsulation is the technology of packing solids or liquids in small packages that release their contents at controlled speeds under specific conditions. The microcapsule is a thin, semipermeable sphere with a tight membrane of a size of a few microns to a millimeter that protects its internal materials. Microencapsulation of probiotics in hydrocolloid substrates can be adopted as a novel method to improve their survival in food during the process, storage, and conversion into powder form for ease of use. This technique is targeted to different types of dairy products such as yogurt, ice cream, milk powder. The microcapsule may be designed for the controlled release of its contents. The factors that cause to break the microcapsule are heat, solubility, diffusion, and pressure and the coating may be designed to release in a specific part of the body. The microcapsule should pass the acidic conditions of the stomach without destruction and open in the intestine [2, 11, 18, 20, 37]. There are several techniques to entrap probiotic bacteria and convert them into powder including spray-drying, freeze-drying, electrospray but these techniques have some disadvantages and limitations which includes expensive equipment, reducing the number of live bacteria after the process, and high production costs [21].

Different materials were used in the encapsulation and entrapment of probiotics including carbohydrates, proteins, and lipids [21]. Milk proteins concentrate (MPC) is produced directly from milk with a combination of

ultrafiltration/diafiltration. MPC can have a protein content range of 55–85% including casein and whey proteins. MPC is additive in many food processes due to high absorbable calcium content, a favorable taste, high water absorption, and ability to increase the viscosity of products. Increasing the efficiency of cheese and yogurt production, formulation of beverages that have high nutritional value are some examples of the use of MPC in the food industry [22, 38].

It seems that a novel strategy is necessary to use probiotics in cereal products due to the disadvantages of common methods of probiotics microencapsulation and the limitations of adding encapsulated probiotics to these foods. There are very few studies on this topic, especially with the use of milk protein concentrate-based films. Therefore, this study aimed to produce edible films containing probiotic bacteria. Hence, MPC-based edible films containing *B. coagulans* IBRC-M 10807 and *L. plantarum* PTCC 1058 were developed. Then the viability of probiotics at different temperatures and storage times was compared and the properties of films were investigated.

## Materials and Methods

### Materials

MPC powder (protein, lactose, moisture, ash, and fat content of MPC were  $84 \pm 1\%$  (w/w),  $3.5 \pm 0.3\%$  (w/w),  $4.0 \pm 0.2\%$  (w/w),  $2.8 \pm 0.1\%$  (w/w), and  $1.1 \pm 0.1\%$  (w/w), respectively) was purchased from Westland New Zealand company. Lyophilized form of *L. plantarum* PTCC 1058 and *B. coagulans* IBRC-M 10807 were obtained from Persian type culture collection (PTCC) and Iranian Biological Research Center (IBRC), respectively. Microbial culture media including De Man, Rogosa and Sharpe (MRS) and Tryptic Soy (TS) broth/agar (TSB/TSA) were purchased from Merck Germany. Glycerol and other chemicals used were laboratory grade and obtained from the Merck Company, Germany.

### Activation of *L. plantarum*

*Lactobacillus plantarum* was inoculated to 10 ml of sterilized MRS broth and incubated at 37 °C for 48 h for reactivation. Then the cultivation was repeated in the 250 ml of MRS broth at same condition. Finally, *L. plantarum* cells were collected by centrifugation at  $5000 \times g$  for 15 min at 4 °C and washed twice by sterile normal saline [24].

### Preparation of *B. coagulans* Spores

*Bacillus coagulans* was added to 10 ml of sterile TSB medium and incubated at 35 °C for 48 h to reactivate the spore of this probiotic. Then it was inoculated to the

250 ml of TSB medium and incubated at the same temperature and time. Bacterial spores were harvested by centrifugation at  $7500 \times g$  for 20 min at 4 °C and washed twice in sterile normal saline. Then purified spores were heated at 65 °C for 45 min to kill any residual vegetative cells. The concentration of spores per ml of spore suspension was verified using the Neubauer chamber and plate count [16].

### Preparation of Probiotic Edible Film

The edible films were made according to the method proposed by Schou et al. [33] with little modification. Briefly, a 10% w/v MPC powder solution was prepared, then different amounts of glycerol (6, 7.5, and 9%w/w) were added according to the experimental design. Then, the bacterial suspension was added (previously set to the half-McFarland standard) to the edible film solution and was stirred for 10 min with a magnetic stirrer. Then the prepared solution was slowly poured into Petri dishes with 12 cm diameter under sterile conditions and dried at 35 °C and 50% relative humidity for 48 h. Finally, the homogeneous films formed were separated from the Petri dishes.

### Enumeration of Probiotic Bacteria

According to the statistical design, viability of the bacteria at different temperatures was monitored during storage. Three circular discs of 1 cm in diameter were cut from each film and stored in sterile plastic bags at –16, 4.5, and 25 °C for 3, 5, and 7 days. On the test day, the disc was placed in a sterile tube and 1 ml of sterile peptone water (0.1% w/v) was added and homogenized with vortex for 1 min until the film was completely dissolved to extract the probiotic bacteria. Subsequent serial dilution was performed in sterile peptone water. *Lactobacillus plantarum* was counted with MRS agar at 37 °C during 72 h of incubation [26]. *Bacillus coagulans* was counted with TSA agar (pH 6.7) at 37 °C for 72 h under aerobic condition [16].

### Characteristics of the Probiotic Edible Films

#### Thickness Measurement

The film thickness measurements were necessary to determine the physical and mechanical properties. A digital micrometer (Mitutoyo-No.293-821, Tokyo, Japan) 0–25 mm was used to measure the thickness with a precision of 0.0001 mm at 5 points (4 points around and one point at center) and the mean was reported for each film [17].

### Measurement of pH

pH of the prepared films was measured by a digital pH meter (781, Mertoem, Netherlands).

### Measurement of Water Solubility

The film samples were cut into  $3 \times 3$  cm squares and dried in an oven at 90 °C until constant weight. Then its weight was measured with an accuracy of 0.001 g and was considered as the initial dry weight of the film. The samples were added in 50 ml distilled water at 25 °C for 6 h under constant stirring (100 rpm) on a shaker. The solution containing the suspended particles of the film was poured onto the paper filter (Whatman No. 5) and dried in an oven at 90 °C until constant weight [17]. The percentage of soluble dry matter was calculated by the following formula:

$$\text{Solubility\%} = (W_1 - W_2) / W_1 \times 100.$$

where,  $W_1$  and  $W_2$  are first constant weight of dried sample (g) and final stable weight of sample (g), respectively.

### Measurement of Moisture Content (MC)

Moisture content of the films was calculated by drying the sample in an oven at 103 °C for 24 h using the following equation:

$$\text{MC(\%)} = (M_i - M_f) / M_f \times 100,$$

where,  $M_i$  and  $M_f$  are the primary and secondary weight of the film sample, respectively [9, 32].

### Measurement of Water Vapor Permeability (WVP)

ASTM E96-00 method at 25 °C was used to determine the water vapor permeability of the films. Prior to measurement, the film samples were conditioned in a desiccator containing saturated magnesium nitrate to reach 52% humidity at 25 °C. The conditioned films were cut into circles with a diameter of 6.5 cm and sealed in vials. Inside the vials was deionized water and there was a gap of about 2 cm between the film and the deionized water. Then, the vials were placed in a desiccator containing silica gel to maintain 0% relative humidity. The weight of the vials was measured at specified intervals and over a specified period of time. The weight loss of the vials will be equivalent to the amount of water passing through the film surface. The weight loss of the vials is recorded over a certain period of time and a chart was drawn. To calculate the water vapor

transmission rate (WVTR), the graph slope is calculated in the linear section and divided by the area of the cup span:

$$\text{WVTR} = \frac{\text{Slope}}{A} = \frac{\Delta m}{\Delta t A}$$

$$\text{WVP} = \frac{\text{WVTR} \times X}{\Delta P}$$

In these equations, the ratio of increasing moisture to time (g/s),  $A$  is the film surface area ( $\text{m}^2$ ) in the test vials,  $X$  is the film thickness (m), and  $P$  is the difference between pure water vapor pressure across the film surface ( $\Delta P$ ) [17].

### Measurement of Color Indexes

Color parameters of the films on the standard screen were measured by the MINOLTA colorimeter. The color value is expressed using Hunter parameters in lightness (L), red-green (a), and yellow-blue (b), and whiteness index (WI) are calculated as follows [14].

$$\text{WI} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

### Experimental Design, Statistical Analysis and Numerical Optimization

In this study, four factors including probiotic type (*B. coagulans* and *L. plantarum*), glycerol (6%, 7.5%, and 9%), temperature (−16, 4.5, and 25 °C), and storage time (3, 5, and 7 days) were investigated by Fractional Factorial design with 22 samples (Table 1). The ANOVA test was used to analyze the obtained results at  $\alpha = 0.05$ . The numerical optimization was performed based on desirability function. The Design-Expert Software Version 10 (Stat-Ease, Minneapolis, MN, USA) was used for statistical design and data analysis.

## Results and Discussion

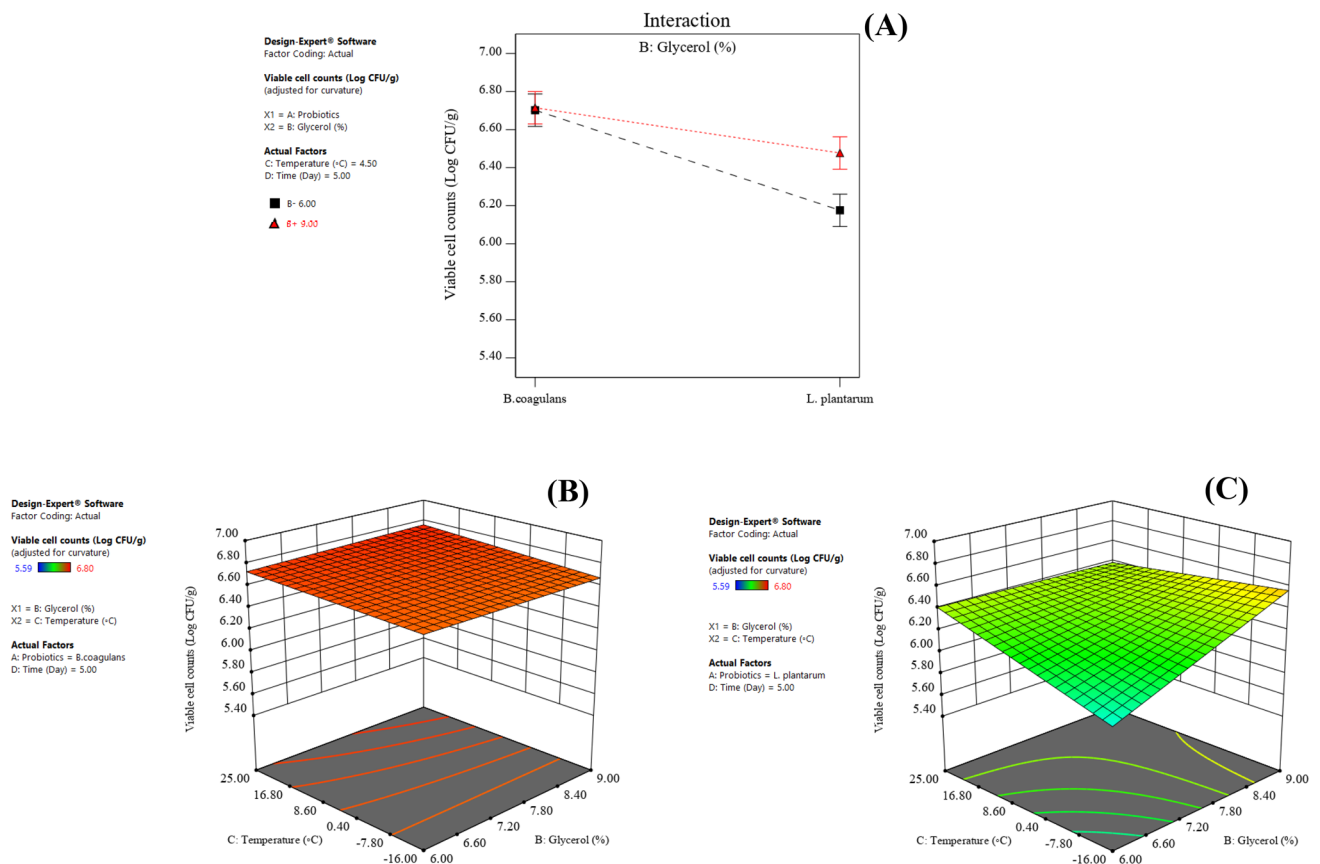
### Survivability of Probiotic Bacteria

The results showed that probiotic type and glycerol concentration had a significant effect on survivability of probiotics ( $p < 0.05$ ). Also, the interaction between probiotic type and glycerol concentration as well as the interaction of glycerol concentration and storage temperature on viability of probiotic bacteria were statistically significant ( $p < 0.05$ ). As can be seen in Fig. 1a, the survivability of *B. coagulans* spores was higher than that of *L. plantarum*. Survivability of *L. plantarum* increased by about 0.30  $\text{Log}_{10}$  CFU/g, upon increasing glycerol concentration from 6 to 9% (Fig. 1a).

**Table 1** Matrix of experimental design

Run	A: Probiotics	B: Glycerol (%)	C: Temperature (°C)	D: Time (day)
1	<i>L. plantarum</i>	7.5	4.5	5
2	<i>L. plantarum</i>	6	25	7
3	<i>L. plantarum</i>	6	−16	7
4	<i>B. coagulans</i>	9	25	7
5	<i>B. coagulans</i>	7.5	4.5	5
6	<i>L. plantarum</i>	9	−16	7
7	<i>L. plantarum</i>	6	−16	3
8	<i>B. coagulans</i>	7.5	4.5	5
9	<i>L. plantarum</i>	9	25	7
10	<i>L. plantarum</i>	9	25	3
11	<i>B. coagulans</i>	9	−16	7
12	<i>B. coagulans</i>	9	−16	3
13	<i>L. plantarum</i>	6	25	3
14	<i>B. coagulans</i>	7.5	4.5	5
15	<i>B. coagulans</i>	6	25	7
16	<i>B. coagulans</i>	9	25	3
17	<i>L. plantarum</i>	9	−16	3
18	<i>B. coagulans</i>	6	−16	7
19	<i>L. plantarum</i>	7.5	4.5	5
20	<i>B. coagulans</i>	6	25	3
21	<i>L. plantarum</i>	7.5	4.5	5
22	<i>B. coagulans</i>	6	−16	3

Figure 1b shows that the viability of *B. coagulans* was almost constant at different studied temperatures. However, survivability of *L. plantarum* with 6% glycerol at 25 °C was about 0.5  $\text{Log}_{10}$  CFU/g higher than that at −16 °C and upon increasing glycerol concentration up to 9%, survivability of this probiotic bacterium at −16 °C was about 0.2  $\text{Log}_{10}$  CFU/g higher than that at 25 °C (Fig. 1c). The decrease in the initial count of probiotic bacteria can be due to the drying of the films at room temperature. Soukoulis et al. [35] reported that WPC significantly improved the viability of *Lactobacillus rhamnosus* GG during drying and storage at room temperature. They also observed a significant decrease in the viability of *L. rhamnosus* GG during the first 24 h of storage. The decrease in viable cell number during the second and third days of storage was low and growth was observed at the last days of storage (days 5–7). The use of protein-based film solutions improved the viability of *L. rhamnosus* GG under simulated gastric and intestinal conditions, and the food matrix had no effect on the inactivation rate [35]. In another study, Soukoulis et al. [36] showed that oligosaccharides and polydextrose significantly increased the survival of *L. rhamnosus* GG during air drying (300% and 75%, respectively), while a decrease by 33% and 80% was observed in the bacterial count for wheat inulin and dextrin. However, inulin had the greatest effect in controlling the



**Fig. 1** Interaction of **a** probiotics and glycerol (%) on viability of probiotics, **b** glycerol (%) and temperature (°C) on viability of *B. coagulans*, c glycerol (%) and temperature (°C) on viability of *L. plantarum*

lethal effects on *L. rhamnosus* GG during storage. In general, addition of probiotics to edible films improved the stability of *L. rhamnosus* GG during storage [36]. Pereira et al. [26] reported that the viability of *Bifidobacterium animalis* BB-12 and *Lactobacillus casei*-01 in whey protein isolate-based edible films was decreased about 3 Log<sub>10</sub> CFU/g after storage at 23 and 4 °C for 60 days. Their results showed that *B. animalis* BB-12 survived a long time with a lower decrease in its cell number in comparison with *L. casei*-01 [26]. Kanmani and Lim [19], reported that pullulan and pullulan/starch films were the most suitable carrier matrix, with maximum viability, 70–80% bacterial cells, after storage at 4 °C for 60 days.

## pH of Films

The results showed that the effect of glycerol concentration and temperature on the pH of probiotic films based on MPC was significant ( $p < 0.05$ ). Figure 2a shows that increase in glycerol concentration resulted in an increase in the pH of probiotic edible films, which could have a positive effect on probiotic viability. The pH of probiotic edible films increased upon increasing temperature from – 16 to

25 °C (Fig. 2b). Sadeghnezhad et al. [30] demonstrated that the pH of film was influenced by the components. According to their results, in protein-based films the amphoteric property of casein protein prevented the increase or decrease of acidity which is not in agreement with findings of this study. This might be due to the presence of lactose in MPC, which causes a slight activity of probiotics in the biopolymer matrix by an increase in glycerol and temperature. Also, with increasing temperature, the side groups of amino acid in MPC might be changed which led to an increase in pH due to increasing hydrogen receptors.

## Solubility of Films

Water solubility is one of the most important properties of edible films. In other words, the solubility of films in water determines the release of antioxidant and antimicrobial compounds from active films when used for food coatings [1]. Resistance to water is an important property of biodegradable and edible films which demonstrates their efficiency in protecting foods with high water activity or fresh and frozen foodstuffs [12]. Shakeri et al. [34] demonstrated that, protein-based films show high water absorption and

**Fig. 2** Effect of **a** glycerol (%) and **b** temperature (°C) on pH of probiotic edible films

Factor Coding: Actual

**pH**

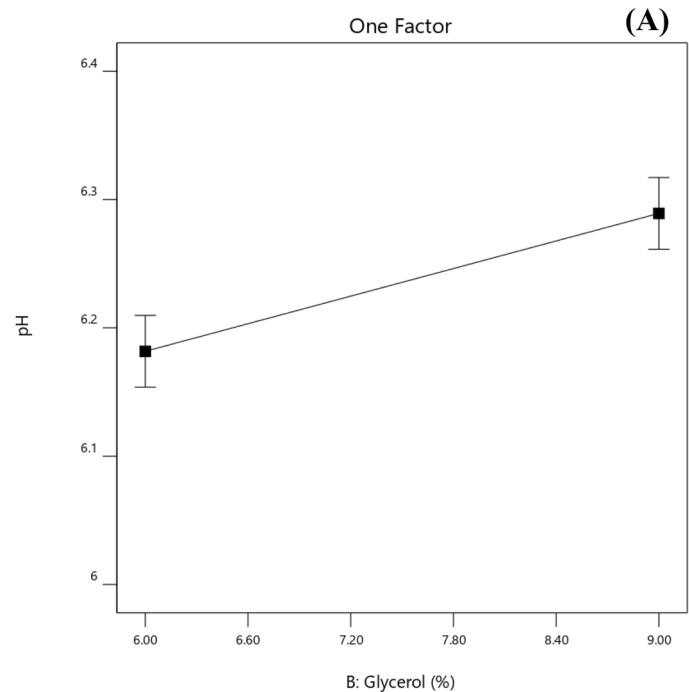
X1 = B: Glycerol (%)

**Actual Factors**

A: Probiotics = B.coagulans

C: Temperature (°C) = 4.50

D: Time (Day) = 5.00



Factor Coding: Actual

**pH**

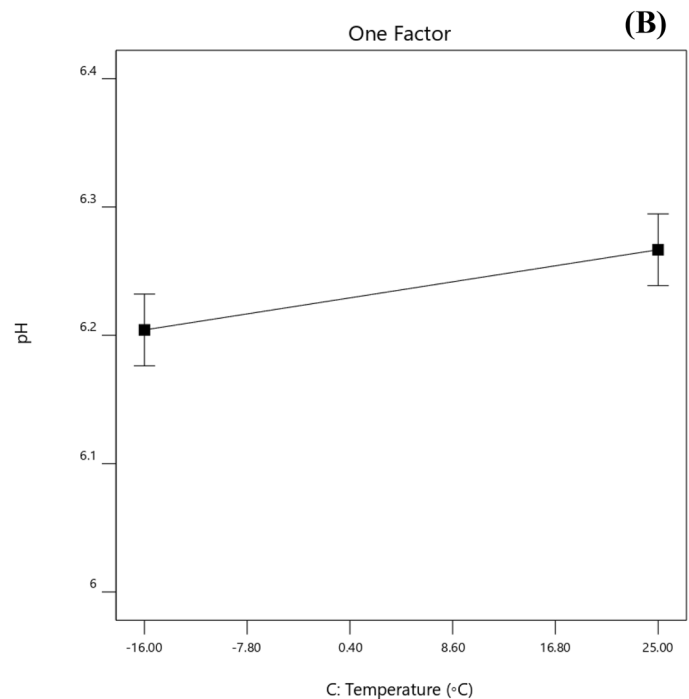
X1 = C: Temperature (°C)

**Actual Factors**

A: Probiotics = B.coagulans

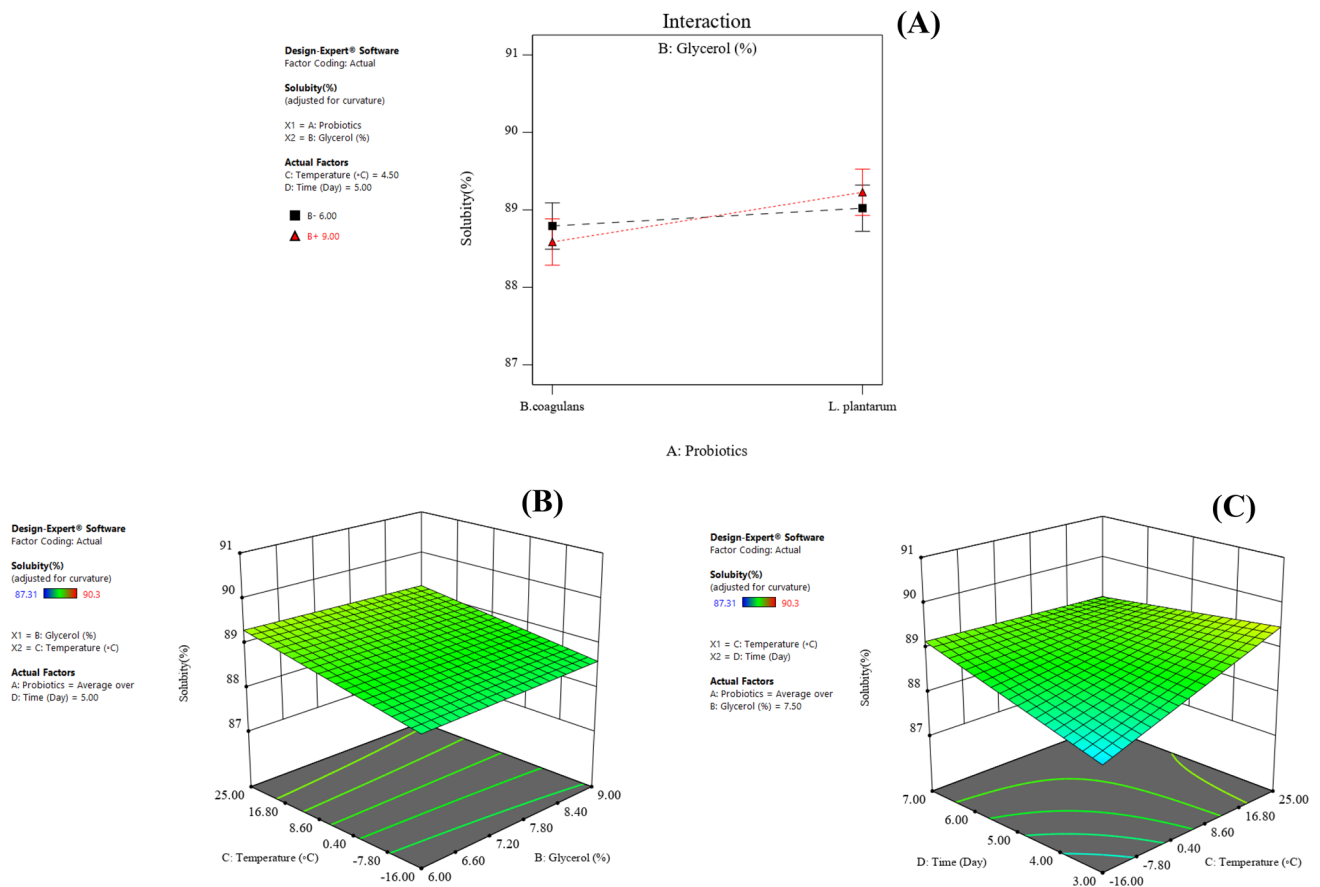
B: Glycerol (%) = 7.50

D: Time (Day) = 5.00



solubility, which limits their application as coating. Usually gum and glycerol due to being soluble in water, increase solubility and lipids due to insolubility, decrease solubility. However, due to interactions between components, gum and glycerol may decrease the solubility of prepared films [39]. The results showed that temperature had a significant effect on the solubility of MPC-based films ( $p < 0.05$ ), as well as the interaction between temperature and time was

statistically significant on the solubility of probiotic edible films ( $p < 0.05$ ). The results showed that, the solubility of the films increased by increasing the storage temperature from  $-16$  to  $25$  °C (Fig. 3a, b). According to this Fig. 3c, the interaction of storage temperature and storage time on solubility, it was increased at  $-16$  °C by increasing time from 3 to 7 days. Solubility was increased by increasing temperature from  $-16$  to  $25$  °C. Chick and Ustunol [12], demonstrated



**Fig. 3** Interaction of **a** probiotics and glycerol (%), **b** glycerol (%) and temperature (°C), **c** glycerol (%) and time (day) on solubility of probiotic edible films

that plasticizers can reduce interactions between (polymer molecules) and increase solubility due to the hydrophilic property of the polymer molecules. Due to the above fact, the effect of the crystallinity and crosslinking between the polymer chains in the molecular structure of the film is very effective on the water solubility of the films [31]. It has been found that by reducing crosslinking between polymer chains, glycerol modifies its molecular structure and significantly increases the solubility of the films in water [12].

### Moisture Content of Films

The results showed that the effect of glycerol and storage time and the interaction between glycerol concentration and temperature on the moisture content (MC) of the films were significant ( $p < 0.05$ ). As can be seen in Fig. 4a, an increase in glycerol concentration increased the moisture content of the films. Increasing storage temperature and time led to decrease the moisture content of the films. The interactions of glycerol (%) and time (day) had significant effect on MC of probiotic edible films and MC was increased by increasing glycerol (%) and time (day) (Fig. 4b). The observed increase


in MC value of films may be attributed to the hygroscopic nature of glycerol, which attracts and holds water molecules during storage time. Therefore, an appropriate selection of the plasticizer type and concentration will be helpful in controlling the MC and, as a consequence, the moisture adsorption rate of a film, thereby improving the film stability under varying RH conditions during storage [30].

### Water vapor permeability of films

Water vapor permeability is a simple way to measure the amount of moisture penetrating and passing through a material. Water vapor transfer from films depends on two factors including solubility and water molecule permeability in the film matrix. This characteristic is one of the important parameters of biodegradable films which is influenced by several factors such as mobility of polymer chains, interaction between functional polymer groups, film structure integrity, hydrophilic to hydrophobic groups ratio and crystalline-to-amorphous region ratio. Based on the results of storage time and the interaction between glycerol concentration and temperature had significant effect on vapor permeability of

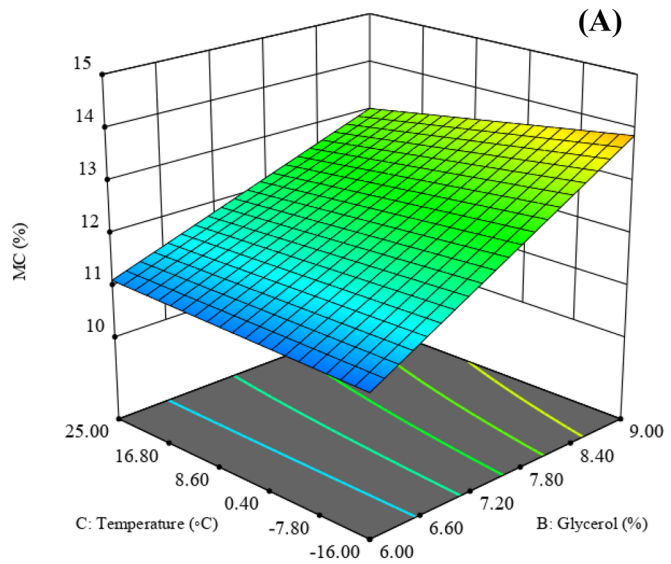
**Fig. 4** Interaction of **a** glycerol (%) and temperature (°C), **b** glycerol (%) and time (day) on moisture content of probiotic edible films

Design-Expert® Software  
Factor Coding: Actual


MC (%)  
(adjusted for curvature)  
10.56  14.58

X1 = B: Glycerol (%)  
X2 = C: Temperature (°C)

Actual Factors  
A: Probiotics = Average over  
D: Time (Day) = 5.00

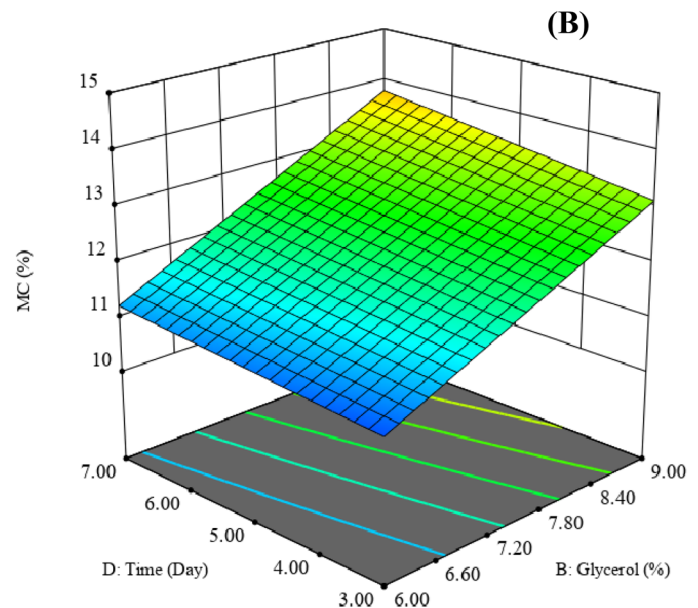


Design-Expert® Software  
Factor Coding: Actual

MC (%)  
(adjusted for curvature)  
10.56  14.58

X1 = B: Glycerol (%)  
X2 = D: Time (Day)

Actual Factors  
A: Probiotics = Average over  
C: Temperature (°C) = 4.50



probiotic films ( $p < 0.05$ ). As shown in Fig. 5a, at 6% glycerol concentration the permeability increased on increasing the storage temperature from  $-16$  to  $25$  °C, but no difference was observed at 9% glycerol concentration. However, at 6% glycerol concentration, water vapor permeability decreased with increasing storage time from 3 to 7 days, but did not differ at 9% glycerol (Fig. 5b). Water vapor permeability of edible films is a main characteristic for the ability of food products to stay healthy against water vapor, especially in dry foods. In fact, one of the main causes of corruption is relative humidity. This is why water vapor deterrence is one of the most important properties of food packaging materials [12]. Food packaging films often need to prevent or at least reduce the transfer of moisture between food and

the surrounding atmosphere. The water vapor permeability should be reduced as far as possible in edible films. In this study the MPC-based probiotic films has very low permeability. The vapor permeability of the films depends on the number of polar groups of the polymer that attracts water molecules to their surface and thus facilitates the water transfer. The higher ratio of hydrophobic compounds causes more inhibition. Edible films based on polysaccharides and proteins have little inhibition against water vapor [12].


### Surface color of films

The results showed that the interaction between probiotic bacteria and temperature had a significant effect on



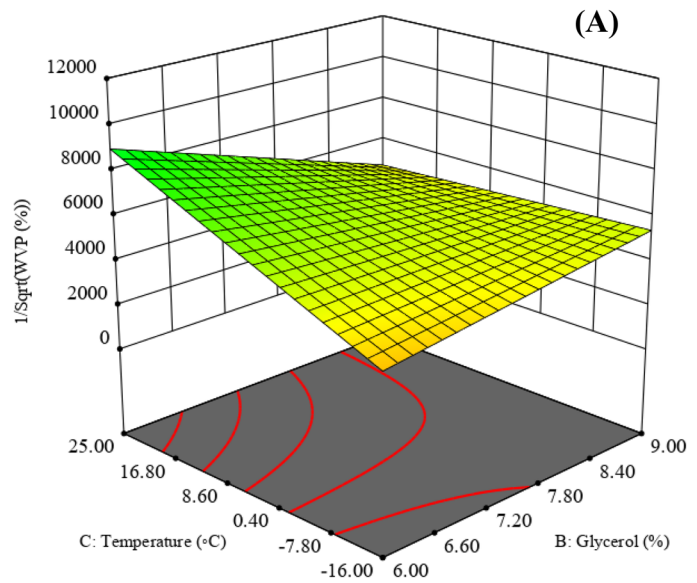
**Fig. 5** Interaction of **a** glycerol (%) and temperature (°C), **b** glycerol (%) and time (day) on WVP of probiotic edible films

**Design-Expert® Software**  
Factor Coding: Actual  
Transformed Scale


**1/Sqrt(WVP (%))**  
(adjusted for curvature)  
15851.1  1019.03

X1 = B: Glycerol (%)  
X2 = C: Temperature (°C)

**Actual Factors**  
A: Probiotics = Average over  
D: Time (Day) = 5.00

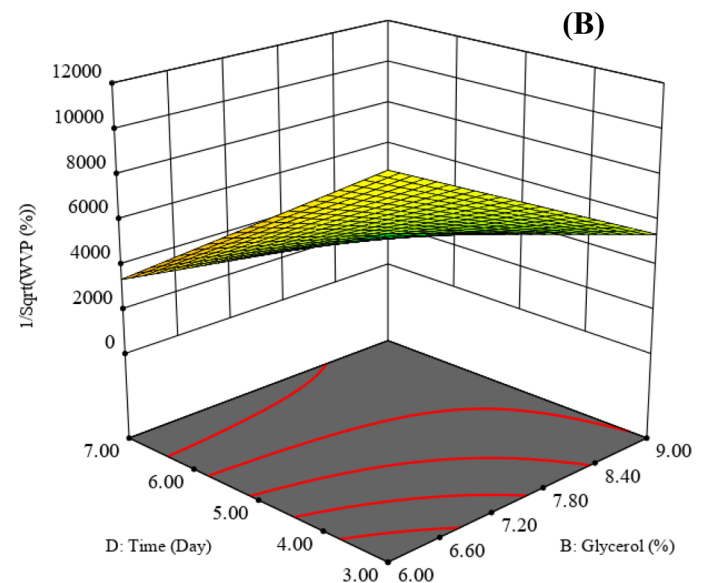


**Design-Expert® Software**  
Factor Coding: Actual  
Transformed Scale

**1/Sqrt(WVP (%))**  
(adjusted for curvature)  
15851.1  1019.03

X1 = B: Glycerol (%)  
X2 = D: Time (Day)

**Actual Factors**  
A: Probiotics = Average over  
C: Temperature (°C) = 4.50



the whiteness index of the films ( $p < 0.05$ ). As shown in Fig. 6, by increasing the temperature, the whiteness index of the *L. plantarum* containing films increased whereas no change was observed in *B. coagulans* containing edible films. This result may be due to differences in size *L. plantarum* cells and *B. coagulans* spores. The spores of *B. coagulans* had non-significant effect on whiteness of edible films due to their much smaller size than *L. plantarum* cells. The color parameters of the films used in food packaging are of great importance because of their effects on consumer acceptability. Higher whiteness index, shows the white color of film [25].

## Numerical optimization of films

Numerical optimization was performed based on desirability function considering maximum probiotic viability, solubility, moisture and whiteness as well as minimum water vapor permeability. The results showed that optimum conditions were 9% glycerol concentration,  $-16\text{ }^{\circ}\text{C}$  and 7 day storage time in which *B. coagulans* viable count was  $6.67\text{ Log}_{10}\text{ CFU/g}$ . The characteristics of optimized probiotic film containing this bacterium were pH 6.25, solubility 89.73%, moisture content 13.58%, WI 44.08 and water vapor permeability  $1.39 \times 10^{-6}\text{ kg m}^{-1}\text{ s}^{-1}\text{ Pa}^{-1}$ . Under the optimum

**Fig. 6** Interaction of a probiotics and temperature (°C) on WI of probiotic edible film

Design-Expert® Software  
Factor Coding: Actual

WI  
(adjusted for curvature)

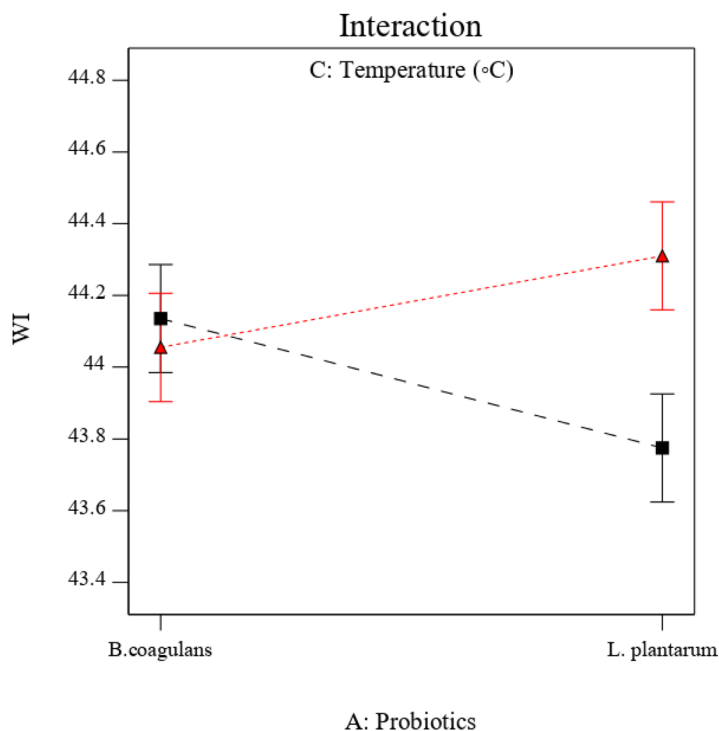
X1 = A: Probiotics  
X2 = C: Temperature (°C)

Actual Factors

B: Glycerol (%) = 7.50  
D: Time (Day) = 5.00

■ C- -16.00

▲ C+ 25.00



conditions, viable count of *L. plantarum* was 6.53 Log<sub>10</sub> CFU/g and characteristics of this probiotic containing edible film included pH 6.25, solubility of 89.98%, moisture content of 14.34%, WI of 44.04 and water vapor permeability of  $4.93 \times 10^{-6} \text{ kg m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ . Five samples of each probiotic film were prepared under the optimized conditions for confirmation of optimization.

## Conclusion

In recent decades, the use of edible films has become one of the most important methods of food packaging. Recent applications of this technique as carriers of bioactive compounds with increased nutritional value have led to their use as carriers of probiotic bacteria. Among the main materials used in the preparation of edible films, proteins due to their special matrix are the major ones. Therefore, this study utilized the incorporation of milk protein concentrate to produce probiotic edible films. Glycerol, on the other hand, is used as a plasticizer in the structure of edible films, which can have a protective effect on probiotics trapped in the film matrix. In this study, in addition to introducing edible film with new formulations, it was used as a carrier for releasing beneficial bacteria with health effects. The results of present study indicated the suitability of the edible film matrix based on milk proteins as a carrier for probiotics. The results of this study showed that both probiotics used had high viability in edible film. Both bacteria had high viability at low

temperatures (−16 °C) due to the presence of glycerol in the edible film structure.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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