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# Survival of probiotic bacteria in carboxymethyl cellulose-based edible film and assessment of quality parameters

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ABSTRACT

In this study, survival of four probiotic strains (*Lactobacillus acidophilus, L. casei, L. rhamnosus* and *Bifidobacterium bifidum*) immobilized in edible films based on carboxymethyl cellulose (CMC) and physicochemical properties of films were investigated during 42 days of storage at 4 and 25 °C. Results showed a significant decrease in viability of bacterial cells during 42 days of storage at 25 °C. However, viability of *L. acidophilus* and *L. rhamnosus* were in the range of recommended levels during the storage at 4 °C ($10^7$ CFU/g). Probiotic films caused more water vapor permeability (WVP)and opacity, and less tensile strength (TS) and elongation at break (EB) compared to the control film. However, no significant physicochemical changes were observed among probiotic films containing different strains. Therefore, incorporation of some probiotic strains in edible coats and films could be their suitable carrier at refrigerated temperatures.



## 1. Introduction

Probiotics are live microorganisms added to food products in certain numbers to improve the quality of the consumer health (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011). Some of the health effects associated to probiotic foods include improved gastrointestinal tract health, reduced lactose intolerance symptoms, reduced serum cholesterol levels and modulated immune system (Thushara, Gangadaran, Solati, & Moghadasian, 2016). To benefit from these health effects, the recommended intake count of the probiotics must be greater than $10^7$ CFU/g of product (Gialamas, Zinoviadou, Biliaderis, & Koutsoumanis, 2010; Jankovic, Sybesma, Phothirath, Ananta, & Mercenier, 2010; Soukoulis, Singh, Macnaughtan, Parmenter, & Fisk, 2016). A wide range of detrimental factors due to food processing (osmotic, mechanical and acid stresses) and storage (oxygen level, hydrogen peroxide and water vapor) has been found to reduce the viability of probiotics (Iaconelli et al., 2015 and Jankovic et al., 2010).

One of the newest approaches to improve the survivability of probiotics is immobilization of them in edible films (Altamirano-Fortoul, Moreno-Terrazas, Quezada-Gallo, & Rosell, 2012). The term “edible films” can be defined as a thin layer of natural polymers directly used onto the surface of materials which can be used to partly or totally substitute synthetic polymers for coating on foods or serving as a barrier between foods and the surrounding environment (Emmambux & Stading, 2007 and Gómez-Guillén et al., 2009). The films protect the food products from deterioration and improve food quality due to roles of moisture barrier and additive carrier. Therefore, they have been recommended as potential vehicles for the delivery of functional compounds (Emmambux & Stading, 2007;; Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). Generally, edible films and coats are classified into three categories based on the components: 1) hydrocolloids such as proteins, polysaccharides and alginates, 2) lipids such as fatty acids, acylglycerols and waxes, and 3) composite films (Garavand, Rouhi,

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Razavi, Cacciotti, & Mohammadi, 2017; Jridi et al., 2014; Skurtys et al., 2010).

Cellulose is the most widely used polysaccharide and the structural component of the cell wall associated with the structural integrity of the cell (Zugenmaier, 2006). The most important limitation of cellulose applications in food technology is water insolubility. Carboxymethyl cellulose (CMC) is one of the water soluble cellulosic derivatives with a wide variety of applications in food and non-food products; such as viscosity modifiers, lubricants, papers, pharmaceutical applications and edible films (Biswal & Singh, 2004).

In this study, the survivability of probiotics and physical and mechanical properties of the probiotic edible films based on carboxymethyl cellulose were investigated.

## 2. Materials and methods

### 2.1. Materials and probiotic bacterial strains

Strains of *Lactobacillus acidophilus, L. casei, L. rhamnosus* and *Bifidobacterium bifidum* were purchased as freeze-dried cultures from TakGene (Tehran, Iran) and kept at −80 °C until used. Carboxymethyl cellulose (CMC) was supplied by Caragum Parsian (Tehran, Iran). Glycerol, Tween 80 (analytical grade), magnesium chloride ($MgCl_2$), sodium chloride (NaCl), and magnesium nitrate ($Mg(NO_3)_2$ were purchased from Merck (Darmstadt, Germany).

### 2.2. Preparation of probiotic cells

Probiotic bacteria were individually inoculated in MRS broth (de Man, Rogosa and Shape, Oxoid, Basingstoke, UK) and then incubated at 37 °C for 48 h. Cell suspensions were transferred to 50 ml sterile tubes under aseptic conditions and centrifuged at 4000 g for 10 min. The supernatant was discarded and the cultured cells were washed twice using phosphate buffer saline (PBS with pH 7.0). The suspension were directly added to film forming solutions (De Lacey, López-Caballero, Gómez-Estaca, Gómez-Guillén, & Montero, 2012).

### 2.3. Preparation of probiotic CMC film

Film solutions were prepared as described by Dashipour et al. (2015) with minor modifications. Solution (1% w/v) was prepared by the gradual addition of 1 g CMC powder to 100 ml distilled water at 70 °C. Solution was mixed well using magnetic stirrer at 500 rpm on for 40 min to ensure uniform dispersion. Then, glycerol (50% of CMC weight) as plasticizer was added to the solution and stirred at 70 °C for 20 min. Solution was heated to 80 °C for 10 min to kill potential pathogens. Air bubbles were removed from the solution by vacuum. When the solution temperature was cooled down to 37 °C, *L. acidophilus, L. casei, L. rhamnosus* and *B. bifidum* were added to film forming solutions to reach a final concentration of $10^9$ CFU/ml. Films were formed by casting 50 ml of the final solutions in the center of sterile glass plates and drying at 35 °C for 15 h in a ventilated incubator. Then, the films were peeled off and stored in zipped bags. Non-probiotic films were prepared as control.

### 2.4. Survival of probiotics in film forming solutions and films

The viability of *L. acidophilus, L. casei, L. rhamnosus* and *B. bifidum* incorporated into the film forming solutions or films was based on a method proposed by De Lacey et al. (2012) with slight modification. Briefly, 1 ml of the solution was suspended in sterile PBS and vortexed for 30 s and then appropriate dilution series were prepared. For the films and before preparing dilution series, 1 g of film was transferred to 99 ml of sterile PBS and mixed gently by constant agitation in a shaker incubator at 37 °C for 1 h to release the bacteria. The serial dilutions were cultured on MRS agar and incubated at 36 °C for 72 h. Enumeration of the bacteria on agar plates was carried out in triplicates using colony count technique (Champagne et al., 2011). The total count of viable bacteria was expressed as log colony forming units per gram (log CFU/g, CFU/g = CFU/plate × dilution factor).

### 2.5. Physical properties of films

#### 2.5.1. Thickness

The thickness of edible films was measured using micrometer with an accuracy of 0.001 mm (Mituto, Tokyo, Japan). At least eight measurements were randomly carried out from different segments of the film and the average values were represented as the film thickness to ensure results consistency.

#### 2.5.2. Moisture content

The moisture content was assessed according to AACC method 44–1502. Pre-weighed aluminum pans containing edible films (approximately 0.7 g) were dried at 105 °C in hot air oven until they reached to constant weight. The moisture content was calculated using the following equation:

$$\text{Percentage of residual water content} = \frac{w_i - w_f}{w_i} \times 100$$

where, $w_i$ and $w_f$ are the initial and final weight of the edible films, respectively.

#### 2.5.3. Color characteristics

Color characteristics of the films were assessed using Hunter lab colorimeter (Reston, USA). The CIE Lab color scale was used to assess $L^*$ (black to white), $a^*$ (red to green) and $b^*$ (yellow to blue) parameters. The total color difference ($\Delta E^*$) between the films and standard color plate was calculated using the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where, $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ are the luminosity, redness and yellowness intensity difference from the standard color plate. All measurements were performed in triplicates.

#### 2.5.4. Opacity

The opacity of films was evaluated based on a method described by Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, and Fisk (2014a). Edible Film specimen were cut into rectangle pieces and directly placed on plastic cuvettes. Absorbance was measured at 550 nm using spectrophotometer (BIOMATE-3S, Thermo Scientific, Waltham, MA, USA). An empty test cell was used as blank. Each film was assessed in three replicates. Film opacity was calculated using the following equation:

$$\text{Opacity} = \frac{A550}{thickness}$$

#### 2.5.5. Water vapor permeability (WVP)

WVP of the films were assessed based on ASTM E96 gravimetric method. Film samples were attached tightly to the top of a cup filled with anhydrous calcium chloride using paraffin wax. The system was placed in a desiccator containing saturated sodium chloride solution (RH = 75 ± 1%) and kept at 25 °C. Weight changes in test cups were recorded periodically with an accuracy of