Iranian Food Science and Technology Research Journal
Vol. 15, No. 3, Aug. Sep. 2019, p. 13-27

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 15، شماره 3، مرداد - شهریور 1398، ص. 27-13

Preparation of anti-Pseudomonas bioactive films by embedding *Lactobacillus casei* ATCC 39392 in Sodium caseinate and Methyl cellulose matrices

H. Ghayoomi[1], M. A. Najafi[1*], N.Soltani Tehrani[1], M. Rahnama[2]

Received: 2017.12.06
Accepted: 2018.11.19

Abstract

Biodegradable films containing lactic acid bacteria (LAB) are considered as new tools for advanced methods of food storage. In this study, *Lactobacillus casei* ATCC 39392 (*L. casei* 39392**)** was directly incorporated into a film formation solution of sodium caseinate (NaCas) and methyl cellulose (MC). The bioactive films were prepared in a manner to contain 10^6CFU/cm^2 *L. casei* 39392. The moisture content, solubility in water, water vapor permeability (WVP), color, opacity, tensile strength, percentage of elongation at break, and the elastic modulus of the films were studied. The survival rate of *L. casei* 39392 was examined during 30 days of storage (5 °C, RH 75%) and the films inhibitory effect on the growth of *Pseudomonas aeruginosa* PTCC 10832 was also studied at 5 °C for 12 days. The presence of *L. casei* 39392 increased the film's opacity and its WVP compared to the control ($p < 0.05$). The survival rate of *L. casei* 39392 was higher in NaCas films than in methylcellulose films ($p < 0.05$). A higher inhibitory effect on the growth of *P. aeruginosa* 10832 (85.3%) was observed in the MC bioactive film, and this inhibitory effect became noticeable from the fourth day of storage onwards ($p < 0.05$). Our results showed that the bioactive films containing *L. casei* 39392 could be used and recognized as biofilms containing natural preservatives.

Keywords: Anti-Pseudomonas, Edible film, *Lactobacillus casei*, Methyl cellulose, Sodium caseinate

Introduction

The development of food storage methods is given an ongoing attention by researchers. So far, various methods have been proposed such as freezing, drying, vacuum packaging and the use of antimicrobial preservatives for food storage; however, some of those methods cannot be used in certain groups of food such as ready-made meals (Quintavalla & Vicini, 2002). Spoilage by microorganisms is a major concern in the realm of food preservation. Bacteria such as *Listeria monocytogenes*, *Pseudomonas spp.* and *Escherichia coli* can cause food spoilage, and their presence in food is often a critical issue when considering the storage of food products (Angiolillo et al., 2014). *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen which mainly targets patients with hematological malignancies (Neves *et al.*, 2014). *P. aeruginosa* is also reported to be the major cause of contamination in poultry, fish, beef and dairy products (Van Tassell *et al.*, 2012). Previous research has shown that the direct use of free antimicrobial compounds in foods result in an immediate but short-term reduction of bacterial populations (Quantavalla & Vicini, 2002). Therefore, a new method was considered, known as active packaging. In this packaging technique, antimicrobial agents are placed inside the film matrices. As a result, antimicrobial effects are maintained for a longer period of time. (Sozer & Kokini, 2009). The duration of antimicrobial activity in the film depends on how antimicrobial compounds are released from the film and how they react with the film's matrix (Leonard *et al.*, 2014). Biodegradable and edible films generally receive greater attention than synthetic films, because they do not end up as waste material, and therefore they exist as environmental friendly (Sozer & Kokini, 2009). Recently, the use of lactic acid bacteria (LAB) in edible films has been considered (Lopez de Lacey *et al.*, 2014). Several reports confirm the effect of metabolites produced by lactic acid bacteria,

1. Food science and technology department, Zabol University.
2. Health and food quality control department, Zabol University.

(*-Corresponding Author Email: najafi413@yahoo.com)
DOI: 10.22067/ifstrj.v15i3.69218

such as the production of bacteriocin-like inhibitory substances (BLIS) (Galvez, Abriouel, & Ben Omar, 2007); organic acids and hydrogen peroxide (Tahiri *et al*., 2009) which inhibit the growth of bacterial pathogens. A well known probiotic LAB that can produce antimicrobial compounds is *Lactobacillus casei* (*L. casei*). Pervious studies have shown that *L. casei* significantly reduce the growth of pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *P. aeruginosa* (Sunaryanto *et al.*, 2014; Erddougrul & Erbilir 2006)

There are numerous reports regarding the use of methyl cellulose (MC) and sodium caseinate (NaCas) biopolymers in the production of edible films (Jimenez et al., 2013; Noronha *et al.*, 2014). Methyl cellulose and NaCas films are biodegradable, inexpensive and easily available. Their ability in preventing the permeation of naturally occurring gases in the atmosphere makes them more applicable (Matsakidou *et al.*, 2013).

Previous studies have also shown that the chemical composition of the matrix can affect the antimicrobial and physical properties of bioactive films (Kanmani and Lim, 2013; Lopez de Lacey *et al*., 2014).

Odila Pereira *et al.* (2016) stated that the viable number of *LAB* decreased only three logarithmic cycles in 60 days in edible films containing whey protein. In a study, *Lactobacillus plantarum* GG ATCC 53103, *Lactobacillus reuteri* ATCC 55730 and *Lactobacillus acidophilus* DSM 20079 were embedded in matrices made of pullulan and various starches. LAB caused slight changes to the mechanical and physical properties of the bioactive films. Also, pullulan and pullulan/potato starch were found to be effective in shaping the aforementioned properties of the films (Kanmani &Lim, 2013).

The first aim of this study was to prepare a new edible film with anti-pseudomonas properties by embedding *Lactobacillus casei* ATCC 39392 (*L. casei* 39392) in a carbohydrate based matrix (methyl cellulose, MC) and in a protein based matrix (NaCas). The second aim was to understand how such embedding can affect the films qualities and structures, including their physicochemical and mechanical properties, and the survivability of the *L. casei* 39392 in the films. Measurements spanned the 30 days of storage (at 5°C and 75% relative humidity). The antimicrobial activity of films against *Pseudomonas aeruginosa* PTCC 10832 (*P. aeruginosa* 10832) was investigated invitro.

Materials and Methods

Propagation of bacterial cells

L. casei ATCC 39392 and *Pseudomonas aeruginosa* PTCC 10832 (*P. aeruginosa* 10832) were purchased in lyophilized form from credible institutions. Lyophilized *L. casei* 39392 was inoculated in 20 ml de Man Rogosa Sharpe Broth (MRS Broth, Liofilchem, Italy) in sterile conditions, and then it was incubated (at 30°C for 24 hours) (Vescovo *et al*., 2006). The cells of *P. aeruginosa* 10832 were transferred to the culture medium of Tryptic Soy Broth (TSB) for 48 hours at 37°C which was then incubated under sterile conditions (Tyagi and Malik, 2011). Microbial suspensions were centrifuged (Model Eppendorf 5810, Germany) for 15 minutes at 3500 g, and the plate was obtained after being washed twice with phosphate-buffered saline (PBS, Merck, Germany) pH=7 to obtain microbial suspensions. Thereafter, the population growth curve for each group of cells was plotted using the serial dilution and pour plate method. The absorbance value of each aliquot was measured using the UV-VIS spectrophotometer device (Cecil model, England) at 600 nm.

Preparation of films

Four grams of the relevant powder (CAS 9004-67-5, Sigma-Aldrich, Madrid, Spain) was dissolved in 100 ml of sterile distilled water at 25 °C in order to prepare the methyl cellulose film, according to Sanchez-Gonzalez et al. (2013). Glycerol was used at 25% w/w, based on its polymer weight (Merk, Germany). The glycerol was added to the film formation solution (FFS) as a plasticizer. It was homogenized in a rotor-stator ultraturrax (DI25, Germany) at 13500 rpm for 4 min. In

order to remove the unwanted cells, the FFS was heated to 80°C for 30 minutes and then quickly cooled to 5°C. By the assistance of a vacuum pump (Jencons, England), degassing was carried out at room temperature.

The NaCas film-forming solution was prepared according to the procedure of Broumand *et al*. (2011) with slight modifications. Five grams of NaCas powder (CAS 9005-46-3, Sigma-Aldrich, Madrid, Spain) was added to 100 mL of sterilized distilled water containing glycerol (30% w/w, based on its polymer weight) at a temperature of 65 ± 5°C in order to prepare the FFS. After complete dissolution, FFS was stirred for 1 hour at 85 ± 2 °C and a vacuum pump was used for its degasification. By transferring 10 µl of *L. casei* 39392 broth culture into 10 mL of MRS broth, bioactive films were became ready to be incubated at 30°C for 24 h. LAB were processed and obtained after being centrifugation at 3500 rpm for 20 min. Reassurance was made to wash the pellets twice with PBS at pH= 7.0 accordingly. Aliqoutes of 240 and 300 µl per 98 ml, respectively of *L. casei* 39392 was then added into the FFS of MC and NaCas-based films. The solution was then mixed on a magnetic stirrer for 5 minutes, and it was endeavored to bring the concentration of *L. casei* 39392 in dry films to 10^6 CFU/cm^2. The FFS of bioactive MC (bio-MC) and bioactive NaCas (bio-NaCas) were transferred to plastic dishes and were dried in an oven at 25 °C for 24 hours. All steps were carried out by using the technique of aseptic and sterile conditions. The average thickness and the total density of the film samples were 0.071 ± 0.05 and 52.2 g/m^2, respectively. In order to adjust moisture (to constant weight), all films were positioned in the desiccator for seven days at 5 °C and at a relative humidity (RH) of 75% before carrying out the mechanical and physical examinations. The 75% RH was reached with the help of NaCl solution that was ultra-saturated.

Survival rate of *L. casei* 39392

The survival rate of *L. casei* 39392 was quantified according to the method used by Gialamas *et al.* (2010) with slight modifications. Bio-NaCas and bio-MC films were placed inside plates and were then collectively placed in the desiccator which had 75% relative humidity at 5°C. This system was maintained for 30 days. A container having saturated salt (NaCl) was used inside the desiccator which caused the moisture content reach 75%. This RH was monitored by a digital hygrometer device (Samwon, South Korea). Every 10 days, the bioactive films were transferred to the Stomacher blender bag (Interscience French model) containing 100 mL of phosphate buffered saline which was then homogenized for 2 minutes. Thereafter, 1000 µl of the sample were removed and, after preparing serial dilutions, it was cultured on MRS agar. The plates were incubated at 30°C for 48 h. Lactobacillus Survival rate was calculated according to the following below formula:

$$\text{Survival rate } (\%) = (N_t / N_0) \times 100 \quad (1)$$

Where N_0 represents the initial number of *L. casei* 39392 and N_t represents the number of alive *L. casei* 39392 in every 10 days of storage**.**

Evaluation of antimicrobial characteristics of bioactive films

From the microbial suspension, aliqout of 50 µl *P. aeruginosa* 10832 was transferred and cultured on the TSA medium. Infusion was particularized in a manner that set the final concentration of *P. aeruginosa* at 10^2 CFU/cm^2 on the medium. Then, films of the control group and the bioactive films were separately placed on the TSA which had been inoculated with the target pathogen. Collectively, this system was incubated at 5°C for 12 days. Under aseptic conditions, samples were transferred to Stomach bags with 100 ml of PBS. Samples were shaked in Stomacher device (interscience, France) for two minutes and serial dilutions were then prepared. Aliqout of 100 µl of the sample was transferred to the TSB and MRS media in order to count the *P. aeruginosa* 10832 and *L. casei* 39392, respectively, in accordance with the procedure described in

section 2.1. The inhibitory effect on the cells' growth rate was calculated in relation to the initial population of cells (Zheng and Zhu, 2003).

$$\text{Inhibition of growth rate } (\%)=((N_0-N_T)/N_0) \times 100 \quad (2)$$

Where: N_0, is the initial number of target bacteria colonies and N_T is the final number of pathogenic bacteria colonies in every 4 days of storage.

Characterization of films

Moisture content

Determination of moisture content of films was performed based on the method introduced by Kurek *et al.* (2014) with some modifications. Fifty mg of the film sample was placed inside the oven at 60°C for 24 hours. Then, the sample was weighed again and, finally, the moisture content of the film was calculated.

Measurement of film solubility in water

Film samples (with the dimensions of 2 × 3 cm) were dried at 60°C for 24 hours and were subsequently weighed. The procedure was followed according to the method proposed by Pinottia *et al.* (2007). Film samples were soaked in 80 ml of water and were then stirred for 1 hour. Finally, the film's insoluble pieces were separated and dried at 60 C overnight. The film's percentage of solubility was calculated according to the following equation.

$$\text{Solubility } (\%)=((W_I-W_F)/W_I) \times 100 \quad (3)$$

Where: W_I is the initial weight of the film and W_F is its weight after the solubility test. Measurements were performed in triplicate.

Water vapor permeability (WVP)

The WVP of the film was evaluated based on the ASTM method E96-95 with some modifications described by Vargas *et al.* (2011) whereby 10 ml of distilled water was added into the glass vial (with an outer diameter of 2 cm), and the film samples were attached firmly to the vials with the help of parafilm. Then, the prepared vials were placed inside the desiccator with a relative humidity of 75% at 5°C. Water vapor transmission was measured according to the vials' weight loss which was measured every 12 hours by using a balance with 0.0001 precision (AND GF-300 model, Japan).

The gradient curve of the weight loss was calculated in relation to time and was plotted over the area (A, m^2) of the film to calculate the water vapor transmission rate (WVTR). WVP of the film was calculated by the following formula.

$$WVP= WVTR \times d/ \Delta P \quad (4)$$

Where d is thickness (mm) and Δp is water vapor pressure difference (Kpa) (Beristain-Bauza *et al.*, 2016).

Color

The colorimetric features of films were evaluated by using the Hunter lab system (Colorimeter, Minolta CR-400, Japan) and values of L (white– black+), a (red– green+) and b (yellow– blue+) were determined. Color differences of each film with the standard sample (Films made of pure biopolymer) were calculated according to the following formula.

$$\Delta E= [(L-L^*)^2+ (b-b^*)^2 + (a-a^*)^2]^{1/2} \quad (5)$$

Where L, a, b are the color parameters of the films formulated with *L. casei* 39392 and L*, a*, b* are the color parameters of the pure film (without lactic acid bacteria) (Beristain-Bauza et al., 2016).

Opacity measurement

The opacity of films was measured according to Núnez-Flores *et al.* (2012) with slight modifications. Firstly, the film was cut into a rectangular shape (0.7×1.5 cm^2) and placed carefully within the spectrophotometer quartz cell. Then, the absorbance value of the film was measured by using a UV-VIS spectrophotometer device (Cecil model, England) at 600 nm, and the air was used as blank for calibration, so that all films were measured in triplicates. The opacity of various films was calculated according to the following formula.

Opacity= A/X (6)

Where: A is absorption at 600λ and X is film thickness.

Mechanical properties

Mechanical tests included tensile strength (TS), percentage of elongation at break (E%), and elastic modulus (EM) which were evaluated by using a texture analyzer (Testometric, M350-10CT, England). Before the test, moisture of film samples was adjusted to 75% relative humidity at 5°C. Then, they were cut into a rectangular shape with the dimensions of 25.4 × 100 mm according to the Standard ASTM D-882 (ASTM, 2001). Grip separation was set at 50 mm and the cross-head speed was 50 mm per minute (Vargas *et al.* 2011).

Morphological characterization using scanning electron microscopy

First, the samples in the liquid nitrogen were broken and, then, they were mounted onto aluminum stubs via tapes that were double-sided. After coating the films with a thin layer of gold, cross-section images were obtained by an electron microscope (EM3200, KYKY, USA) with an acceleration voltage of 26 kV.

Statistical analyses

Results were analyzed by SAS software version 9.1 using the factorial experiment in a completely randomized design (CRD) with three replications and the comparisons of mean values of the physical, mechanical and biological data were performed with the Duncan and Tukey's tests, respectively. All tests were performed in triplicates.

Results and discussion

Moisture, water solubility and WVP

Moisture, water solubility and WVP of the films are three of the most important factors in edible films that determine the duration of shelf life and the quality of foods. Moisture, water solubility and WVP of the films are shown in Fig. 1 (a, b and c). The NaCas film (10.83±0.81%) had more moisture content in comparison with the MC film (9.67± 0.60%), which indicates that the NaCas film exhibits more hydrophilicity compared to the MC ($p<0.05$).

There was no significant difference in the moisture content (10.83±0.8- 10.90±0.82%) and water solubility (85.17±0.4- 85.62±0.5) when comparing NaCas films and bio-NaCas ($p<0.05$). But the bio-MC film showed the highest moisture content (12.83±0.40%) and the highest water solubility (94.07 ±0.7%) ($p<0.05$).

Under lactose starvation, *L. casei* can change its metabolic pathways and produce catabolic enzymes that enable *L. casei* to use beta-glucosidase, maltose, trihalose and ribose, and this results in structural changes to the polysaccharide polymers; however, the metabolism of proteins and amino acids remained largely unchanged or slightly suppressed during starvation (Naseri *et al.*, 2013).

Piermaria *et al.* (2015) reported that the presence of *Lactobacillus plantarum* and *Saccharomyces marxianus* did not change the moisture content of the Kefiran films. This observation could be due to the difference in the chemical structure of the films and the type of embedded microorganisms. Beristain-Bauza *et al.* (2016) stated that the addition of microbial metabolites to biofilms would increase solubility. There are differences in the ionization of hydroxyl and carboxyl groups and the decomposition of hydrogen and ionic bands which is probably due to the high polarity of film composition or water penetration (Soradech *et al*., 2012). The results showed that the WVP of NaCas and MC films were significantly different which can be related to the hydrophilicity of the NaCas polymer ($P<0.05$). Bio-films (16.85- 19.73 g× mm KPa^{-1} h^{-1} m^{-2}) produced significantly higher WVP compared to pure films (14.09- 17.33 g× mm KPa^{-1} h^{-1} m^{-2}) with similar biopolymers. This parameter is influenced by some factors such as thickness, biopolymer structure, intermolecular reactions, type of softener used and conditions of storage (including temperature and relative humidity) (Bertuzzi *et al*., 2007). The images of bio-films obtained by the SEM (2B and 2D)

show that the presence of *L. casei* 39392 causes discontinuities in the matrices of films, thereby increasing the movability of polymer chains in the transfer of water molecules (Sanchez-Gonzalez *et al*., 2013). This result is not in line with those reported by Gialamas *et al*. (2010), and the previous researcher did not observe significant differences in terms of the barrier properties of the sodium caseinate films when the bioactive medium was added. Kurek *et al*. (2014) stated that the high moisture content of films could cause an increase in WVP. This is probably due to the fact that water molecules react with polar groups of the polymer chains, causing the film's WVP to increase (Slavutsky & Bertuzzi, 2012).

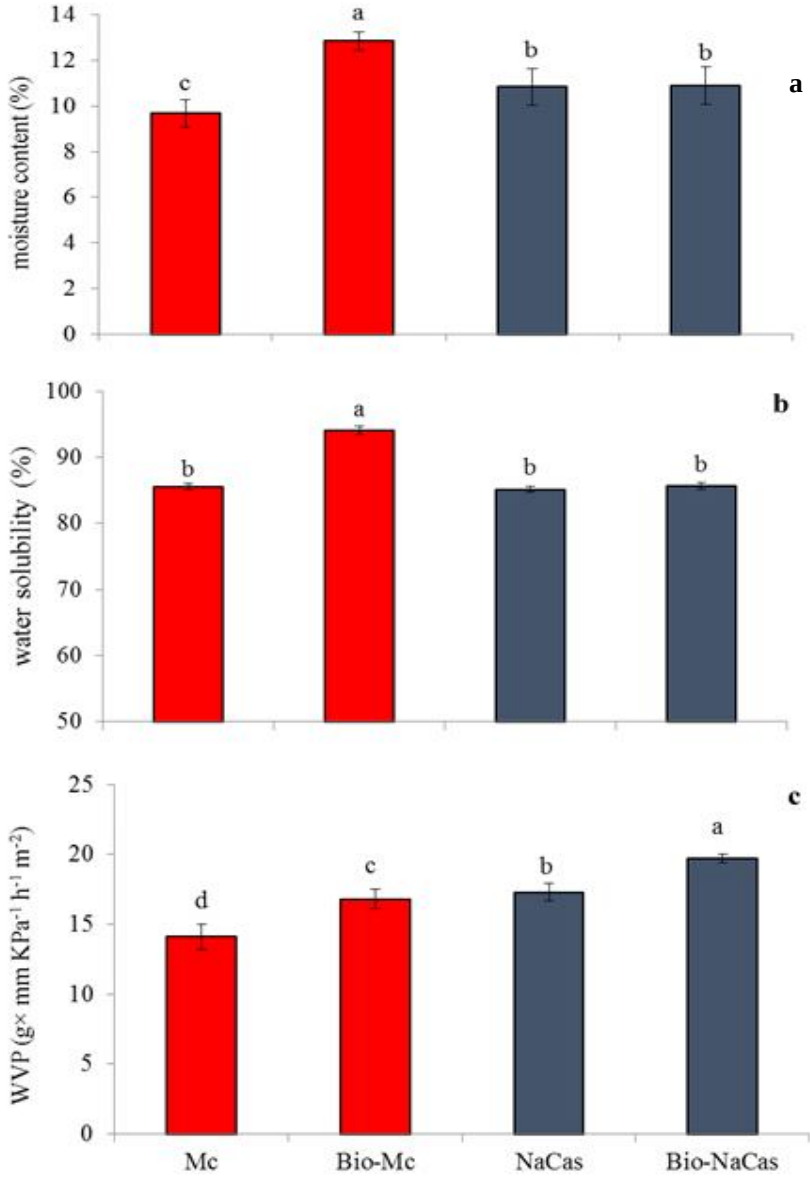

Fig 1. Changes in moisture (a), water solubilty (b) and water vapor permability (WVP, c) of films.
The values are the means± SD of triplicate expriments. [a-d] Difference letters in each column indicate significant differences ($p<0.05$). MC, Methylcellulose; Bio-MC, MC film containing *Lactobacillus casei* ATCC 39392; NaCas, Sodium caseinate; Bio-NaCas, NaCas film containing *Lactobacillus casei* ATCC 39392.

Optical properties

Table 1 shows the mean values of the color and opacity parameters of the films. The results showed that the highest amount of *L* was observed in the Bio-MC. Incorporating *L. casei* 39392 into the film matrix increased the clarity (*L*) of the film. The highest *a* value was observed in the MC film. There was no significant difference among the amount of *a* parameter in the NaCas, Bio-NaCas and Bio-MC films (P>0.05).

Table 1- Changes in optical properties of films

Film	Optical properties				
	L	*a*	*b*	*ΔE*	opacity (A650/X)
NaCas	88.85±0.05^{d}	1.72±0.03 ^{b}	-2.32±0.10 ^{ab}	----	2.50±0.11^{a}
Bio-NaCas	89.05±0.01^{c}	1.74±0.06^{b}	-2.16±0.10^{a}	0.30±0.10^{b}	1.11±0.10 ^{b}
Mc	89.28±0.13^{b}	1.82±0.03 ^{a}	-2.64 ±0.10^{bc}	----	1.21±0.09^{b}
Bio-MC	89.54±0.10 ^{a}	1.69±0.01^{b}	-2.93±0.30^{c}	0.60±0.15^{a}	0.50±0.02^{c}

The values are the means ± SD of triplicate expriments. $^{a-d}$Difference letters in each column indicate significant differences (p<0.05). NaCas, Sodium caseinate; MC, Methylcellulose; Bio-MC, MC film containing *Lactobacillus casei* ATCC 39392; Bio-NaCas, NaCas film containing *Lactobacillus casei* ATCC 39392

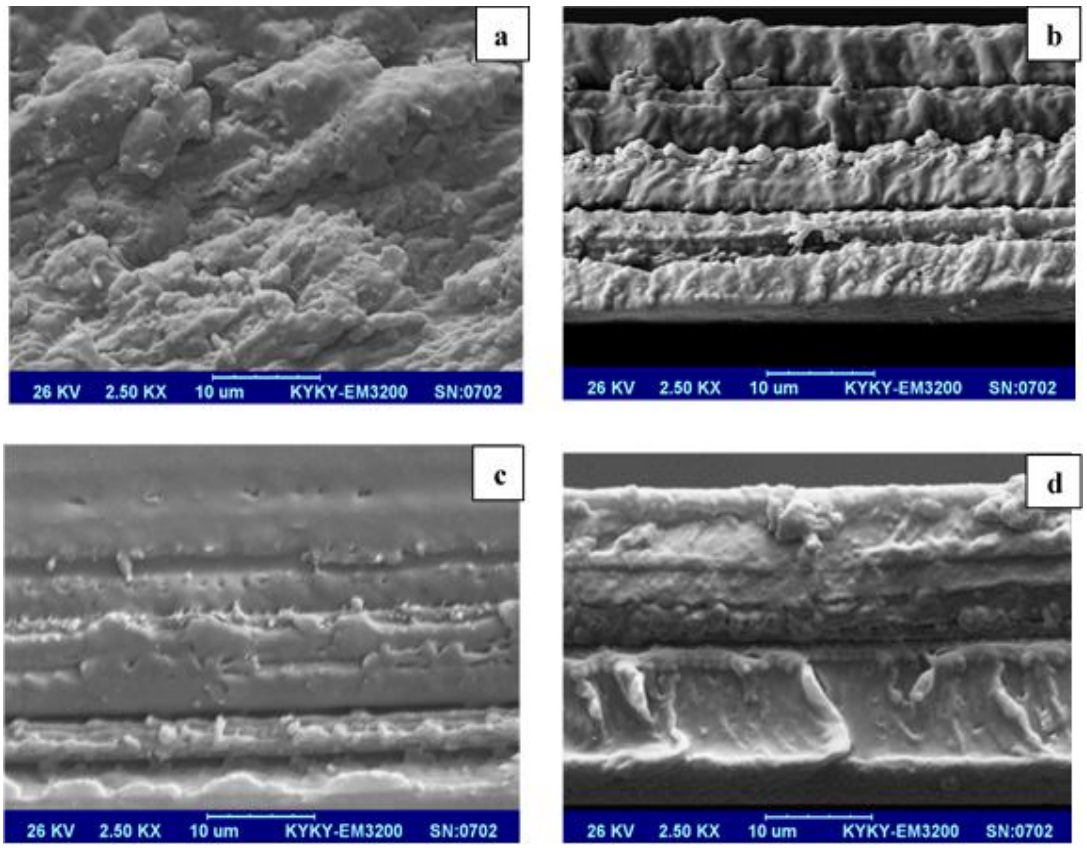

Fig. 2. Scaning electron microscop micrographs of the cross-section of films. a: Methylcellulose; b: Methylcellulose + *Lactobacillus casei* ATCC 39392; c: Sodium caseinate; d: Sodium caseinate *Lactobacillus casei* ATCC 39392

Furthermore, the results showed that there was no significant difference between the *b* values of NaCas and Bio-NaCas films, and also no significant difference between the MC and Bio-MC in that respect, which indicates that *L. casei* 39392 had no significant effect on the parameter *b* (blue-yellow) ($P>0.05$). The results also showed that the ΔE value for Bio-MC was significantly higher than the value for Bio-NaCas. Given that the calculated ΔE is less than 2 units, the resultant color change would not be visible. Beristain-Bauza *et al.* (2016) stated that applying 18 mg/ ml of a cell-free supernatant of *Lactobacillus rhamnosus* in a NaCas matrix and a whey protein isolate can decrease the parameter *L* and increase the parameter *b* and color variation (ΔE), in both matrices. The different results might be due to the difference in the biopolymer and the additive to the film matrix. As Table 2 shows, the MC

Film was more opaque than NaCas ($P<0.05$) which can be due to the more uniform structure of the NaCas film (fig.2c) compared to the MC (fig.2a). The highest value of opacity was observed in the NaCas. Matsakidou *et al.* (2013) stated that the increase in the moisture content of the film could reduce the opacity, while our results indicated that the moisture content of the NaCas and Bio-NaCas films did not differ significantly. However, there was a significant difference in opacity among the films ($P<0.05$). The results showed that the biofilms are more transparent than the pure films with the same matrix, which indicates the significant effect of *L. casei* 39392 on film opacity. Kanmani and Lim (2013) reported that embedding *L. plantarum*, *L. reuteri* and *L. acidophilus* in the matrix of pullulan/starch, tapioca and potato starch could reduce opacity. However, those results do not match the results obtained in this study due to the variation in chemical structures of the films as well as in the microorganisms embedded.

Mechanical properties

The mechanical properties of films were investigated with parameters of TS (MPa), EM (MPa) and E (%). Fig.1 shows that the TS (Fig. 1a), EM (Fig. 1b), and E values (Fig. 1c), in the MC film are higher than in the NaCas film ($p<0.05$). This is probably due to the difference in the chemical composition and the homogeneous structure of the MC film (Fig. 2a) ($p<0.05$). The results show that the mechanical properties of bio-NaCas have no significant difference compared to pure NaCas films, which indicates that *L. casei* 39392 had no effect on the mechanical properties of NaCas-based films ($p>0.05$). Adding *L. casei* 39392 to the FFS of MC served to reduce the TS and EM, but increased the E values compared to pure MC films ($p<0.05$). It has been reported that *Lactobacillus casei* and *Bifidobacterium animalis* Bb-12 were embedded in whey protein isolate matrices which resulted in the reduction of TS but did not change the EM and E% values of the film (Pereira *et al*., 2016). The mechanical properties of films are belived to be influenced by parameters such as the moisture content and the interconnected film matrix. Moisture content acts as a plasticizer, thereby reducing the TS and EM but increasing the E% values (Pittia & Sacchetti, 2008; Aguirre-Loredo *et al.*, 2016). This is consistent with the results obtained in this study.

Survival rate of *L. casei* 39392 in the biofilm

The survival rate (SR) of *L. casei* 39392 in Bio-MC and Bio-NaCas is shown in Fig. 4 for 30 days of storage (5°C and relative humidity of 75%). The results showed that during the storage of *L. casei* 39392, the SR decreased significantly and the lowest SR in Bio-MC ($71.4 \pm 0.6\%$) was observed on day 30 of storage. Romano *et al.* (2014) reported that the SR value of LAB decreased with time in MC films. It was also observed that the viability of *Bifidobacterium animalis* and *L. casei* in whey protein-based films decreased by 1 and 2 logarithmic cycles during storage at 4°C. (Odila Pereira *et al*., 2016). Furthermore, it has been reported that embedding LAB, as a softening compound, in the film accelerates enzymatic and chemical reactions such as the oxidation of the cytoplasmic membrane lipids, and reduces the survival rate (Soukoulis *et al*., 2016).

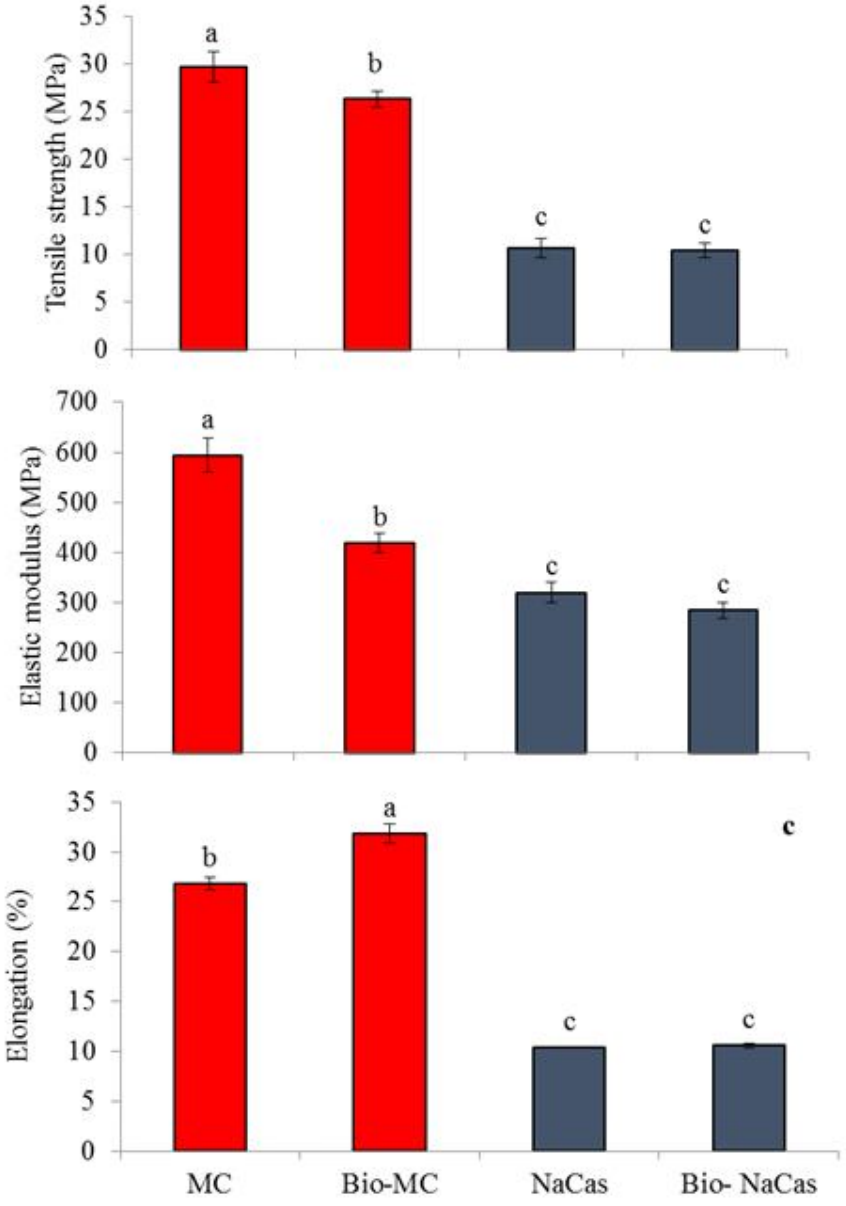

Fig. 3. Changes in tensile strength (a), elastic modulus (b) and elongation at break (c), of films.
The values are the means ± SD of triplicate expriments. [a-c] Difference letters in each column indicate significant differences ($p<0.05$). MC, Methylcellulose; Bio-MC, MC film containing *Lactobacillus casei* ATCC 39392; NaCas, Sodium caseinate; Bio-NaCas, NaCas film containing *Lactobacillus casei* ATCC 39392 .

The Bio-NaCas in this research appeared to have the highest SR compared to the Bio- MC, and this can be confirmed by studying the SR of *L. casei* 39392 embedded in the biofilm matrix throughout the 30 days of storage ($p<0.05$). Previous studies have shown that *Lactobacillus paracasei* and *Lactococcus lactis* have higher survival rates in the alginate-NaCas matrix than in alginate (Leonard *et al*., 2015) which can be due to a higher buffering capacity,

better bacterial placement, nutrient supply (peptide and amino acid) and better control over free radicals by caseinates (Leonard *et al*., 2014). Fu and Chen (2011) stated that the viability rate of LAB depends on the strain of microorganisms, the type of matrix, storage temperature, oxidative stress and film structure.

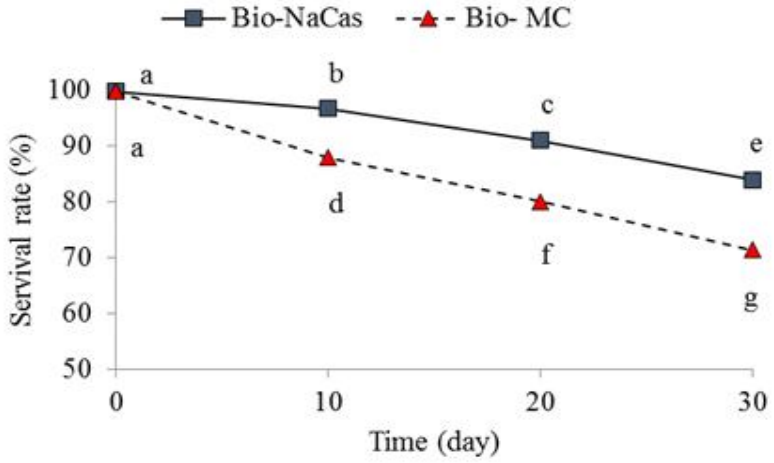

Fig. 4. Survival rate *Lactobacillus casei* ATCC 39392 embedded in films, on the tryptone soya agar medium contaminated with *Pseudomonas aeruginosa* PTCC 10832.

The values are the means of triplicate expriments. [a-g] Difference letters indicate significant differences among films with different formulation at the storage time ($p < 0.05$). Methylcellulose film containing *Lactobacillus casei* ATCC 39392; Bio-NaCas, Sodium caseinate film containing *Lactobacillus casei* ATCC 39392 .

Anti-pathogenic effect of films

The results varied among the Bio-MC and Bio-NaCas films regarding their effects on the growth inhibition of *P. aeruginosa* 10832 (at 5℃ for 12 days of storage) (Fig. 5).

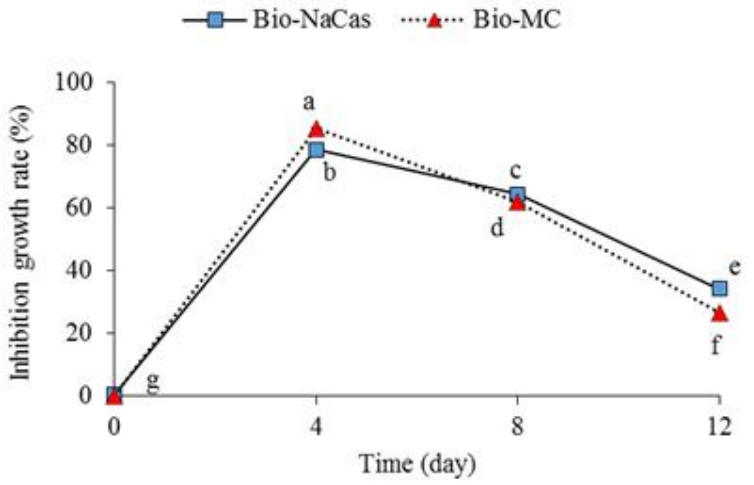

Fig 5. Effects of biofilms containing *Lactobacillus.casei* ATCC 39392 on growth inhibitory and *Pseudomonas aeruginosa* PTCC10832 in the tryptone soya agar medium.

[a-g] Difference letters indicate significant differences among films with different formulation at the storage time (p 0.05).

The results showed that Bio-MC and Bio-NaCas had inhibitory effects on the growth of *P. aeruginosa* 10832. A similar inhibitory effect was observed in the whey protein isolate

and in the calcium caseinate films which contained a cell-free supernatant of *Lactobacillus rhamnosus* (Beristain-Bauza *et al*., 2016).

Bio-MC showed the highest growth inhibition rate against *P. aeruginosa* 10832 (85.3%) on the fourth day. It has been reported that the presence of polysaccharides in an environment can have impacts on the metabolism of the LAB and can increase the production of antimicrobial compounds such as bacteriocin, organic acids and hydrogen peroxide (Galvez *et al*., 2007).

Pure NaCas and MC films showed no inhibitory effects on the pathogens' growth throughout the storage period. Furthermore, the high solubility of the Bio-MC can accelerate the diffusion of antimicrobial compounds. On the eighth and twelfth day of storage, the Bio-NaCas showed a stronger inhibitory effect on the growth of P. aeruginosa 10832, whereas the Bio-MC had a weaker inhibitory effect ($p<0.05$).

Furthermore, with respect to the higher survival rate of *L. casei* 39392 in Bio-NaCas, the inhibitory effect on pathogenic growth was more sustainable in Bio-NaCas up to the end of storage period.

Conclusion

The results showed that the chemical structure of the film affects the viability of the lactic acid bacteria, the intensity of antimicrobial effects, and the changes in the physical and mechanical properties of the film. *L. casei 39392* did not change the mechanical properties of the NaCas film but improved its elongation and reduced the elastic modeulus and Tensile strenght of the MC film by changing the structural of a backbone polymer. It also increased the opacity (41-44%) and the *L* parameter of the films (%0.22-0.26). *L. casei* 39392 was able to survive more when embedded in the NaCas matrix, compared to MC matrix. Ultimately, it can be claimed that the bio-MC film had a stronger anti-pseudomonas effect for up to 6 days of chilled storage. The results of this study demonstrate the anti-pathogenic properties of these bio-films *in vitro*. Our findings engender a new approach to the natural preservation, and may be able to increase the shelf life of some food products such as meat and cheese.

Refrences

Al-Naseri, A., Bowman J. P., Wilson R., Nilsson R. E., & Britz M. L., 2013. Impact of Lactose Starvation on the Physiology of Lactobacillus casei GCRL163 in the Presence or Absence of Tween 80. *Journal of Proteome Research.* 12: 5313-5322.

Aguirre-Loredo, R. Y., Rodríguez-Hernández, A. I., Morales-Sánchez, E., Gómez-Aldapa, C.A., & Velazquez, G., 2016, Effect of equilibrium moisture content on barrier, mechanical and thermal properties of chitosan films. *Food Chemistry*, 196: 560–566.

Angiolillo, L., Conte, A., Faccia, M., Zambrini, A.V. & Del Nobile, M. A., 2014. A new method toproduce synbiotic Fiordilatte cheese. *Innovative Food Science and Emerging Technologies*. 22: 180–187.

ASTM (1995). Annual Book of ASTM Standards. American Society for Testing and Materials, Philadelphia, PA. pp. 406-413.

ASTM **(**2001). Annual Book of ASTM Standards. American Society for Testing and Materials, Philadelphia, PA. pp. 162-170

Beristain-Bauza, S. C., Mani-López, E., Palou, E., & López-Malo, A., 2016, Antimicrobial activity and physical properties of protein films added with cell free supernatant of *Lactobacillus rhamnosus*. *Food Control*, 62: 44–51.

Bertuzzi, M. A., Castro Vidaurre, E. F., Armada, M., & Gottifredi, J. C., 2007, Water vapor permeability of edible starch based films *Journal of Food Engineering.* 80: 972-978.

Broumand, A., Emam-Djomeh, Z., Manouchehr, H. and Razavi, S, H., 2011, Antimicrobial, water vapour permeability, mechanical and thermal properties of casein based Zataraia multiflora Boiss. Extract containing film. *LWT - Food Science and Technology*, 44: 2316-2323.

Erddougrul, Ö. and Erbilir F., 2006, Isolation and characterization of *Lactobacillus bulgaricus* and *Lactobacillus casei* from various foods. *Turkish Journal of Biology*, 30: 39-44.

Fu, N., & Chen, X. D., 2011, towards a maximal cell survival in convective thermal drying processes. . *Food Research International*, 44: 1127-1149.

Galvez, A., Abriouel, H., Lopez, R. L., & Ben Omar, N., 2007. Bacteriocin-basedstrategies for food biopreservation. . *International Journal of Food Microbiology*, 120: 51-70.

Gialamas, H., Zinoviadou, K. G., Biliaderis, C. G. and Koutsoumanis, K. P**.**, 2010. Development of a novel bioactive packaging based on the incorporation of *Lactobacillus sakei* into sodium-caseinate films for controlling *Listeria monocytogenes* in foods. *Food Research International*, 43: 2402–2408.

Jimenez, A., Jose Fabra, M., Talens, P., & Chiralt, A., 2013. Physical properties and antioxidant capacity of starch–sodium caseinate films containing *lipids. Journal of Food Engineering*, 116: 695–702.

Kanmani, P., & Lim, S. T., 2013, Development and characterization of novel probiotic residing pullulan/starch edible films. *Food Chemistry*, 141: 1041–1049.

Kurek, M., Guinault, A., Voilley, A., Galic, K., & Debeaufort, F., 2014, Effect of relative humidity on carvacrol release and permeation properties of chitosan based films and coatings, *Food Chemistry*. 144: 9–17.

Leonard, L., Degraeve, P., Gharsallaoui, A., Saurel, R. & Oulahal, N., 2014, Design of biopolymeric matrices entrapping bioprotective lactic acid bacteria to control *Listeria monocytogenes* growth: Comparison of alginate and alginate-caseinate matrices entrapping *Lactococcus lactis subsp. lactis* cells, Food Control, 37: 200-209.

Leonard, L., Beji, O., Arnould, C., Noirot, E., Bonnotte, A., Gharsallaoui, A., Degraeve, P., Lherminier, J., Saurel, R., & Oulahal, N., 2015, Preservation of viability and anti-Listeria activity of lactic acid bacteria, *Lactococcus lactis* and *Lactobacillus paracasei*, entrapped in gelling matrices of alginate or alginate/caseinate, *Food Control*. 47: 7-19.

Lopez de Lacey, A. M., Lopez-Caballero, M. E. & Montero, P**.,** 2014, Agar films containing green tea extract and probiotic bacteria for extending fish shelf-life. *LWT - Food Science and Technology* 55: 559-564.

Matsakidou, A., Biliaderis, C. G., & Kiosseoglou, V., 2013, Preparation and characterization of composite sodium caseinate edible films incorporating naturally emulsified oil bodies. *Food Hydrocolloids*, 30: 232-240.

Min, S., Harris, L. J., & Krochta, J. M., 2005, Antimicrobial effects of lactoferrin, lysozyme, and the lactoperoxidase system and edible whey protein films incorporating the lactoperoxidase system against Salmonella enterica and Escherichia coli O157: H7 *Journal of Food Science*, 70: 332-338.

Neves, P. R., Mc-Culloch, J. A., Mamizuka, E. M., & Lincopan, N., 2014. *Pseudomonas aeruginosa*. Encyclopedia of Food Microbiology, 3, 253-260.

Noronha, C. M., de Carvalho, S. M, Lino, R. C., Manique Barreto, P. L., 2014, Characterization of antioxidant methylcellulose film incorporatedwith a-tocopherol nanocapsules. *Food Chemistry*. 159: 529–535.

Núnez-Flores, R., Gimenez, B., Fernandez-Martin, F., Lopez-Caballero, M. E., Montero, M. P., & Gomez-Guillen, M. C., 2012, Role of lignosulphonate in properties of fish gelatin films. *Food Hydrocolloids*, 27(1), 60-71.

Odila Pereira, J., Soares, J., Sousa, S., Raquel Madureira, A., Gomes, A., & Pintado, M., 2016, Edible films as carrier for lactic acid bacteria. *LWT - Food Science and Technology*, 73: 543-550.

Piermaria, J., Diosma, G., Aquino, C., Garrote, G., & Abraham, A., 2015, Edible kefiran films as vehicle for probiotic microorganisms. *Innovative Food Science and Emerging Technologies*, 32: 193–199.

Pinottia, A., Garcy, M. A., Martinoa, M. N., Zaritzky, N. E., 2007, Study on microstructure and physical properties of composite films based on chitosan and methylcellulose. Food Hydrocolloids, 21: 66–72.

Pittia, P., & Sacchetti, G., 2008, Antiplasticization effect of water in amorphousfoods. A review. *Food Chemistry*, 106: 1417–1427.

Pothakamury, U. R., & Barbosa-Canovas, G. V., 1995, Fundamental aspects of controlled release in foods. *Trends in Food Science & Technology*, 6: 397-406.

Pranoto, Y., Rakshit, S. K., & Salokhe, V. M., 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. . *LWT - Food Science and Technology*. 38: 859-865.

Quintavalla, S. & Vicini, L., 2002, Antimicrobial Food Packaging in Meat Industry. *Meat Science*, 62: 373-380.

Romano, N., Tavera-Quiroz, M. J., Bertola, N., Mobili, P., Pinotti, A., & Gómez-Zavaglia,

A., 2014, Edible methylcellulose-based films containing fructo-oligosaccharides as vehicles for lactic acid bacteria. *Food Research International*. 64: 560–566.

Salgado, P. R., Lopez-Caballero, M. E., Gomez-Guillen, M. C., Mauri, A. N., & Montero, M. P., 2013, Sunflower protein films incorporated with clove essential oil have potential application for the preservation of fish patties. *Food Hydrocolloids*, 33: 74-84.

Sanchez-Gonzalez, L., Quintero Saavedra, J. I. & Chiralt, A**.,** 2013. Physical properties and antilisterial activity of bioactive edible films containing *Lactobacillus plantarum*. *Food Hydrocolloids*, 33: 92-98.

Schnurer, J., & Magnusson, J., 2005, Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science & Technology*, 16: 70-78.

Slavutsky, A. M., & Bertuzzi, M. A., 2012, a phenomenological and thermodynamic study of the water permeation process in corn starch/MMT films. *Carbohydrate Polymers*, 90: 551–557.

Soradech, S., Nunthanid, J., Limmatvapirat, S., & Luangtana-anan, M. 2012, Anapproach for the enhancement of the mechanical properties and film coating efficiency of shellac by the formation of composite films based on shellac andgelatin. *Journal of Food Engineering*, 108: 94–102.

Soukoulis, C., Singh, P., Macnaughtan, W., Parmenter, C., & Fisk, I**.,** 2016, Compositional and physicochemical factors governing the viability of *Lactobacillus rhamnosus GG* embedded in starch-protein based edible films. *Food Hydrocolloids*, 52: 876-887.

Sozer, N., & Kokini, J. L., 2009, Review nanotechnology and its applications in the food sector. *Trends in Biotechnology*, 27: 82–89.

Sunaryanto, R., Martius dan, E., & Marwoto, B., 2014, Capability test of *Lactobacillus casei* as probiotic agent. *Journal bioteknologi and biosains Indonesia*, 1: 9-15

Tahiri, I., Desbiens, M., Kheadr, E., Lacroix, C., & Fliss, I., 2009, Comparison of different application strategies of divergicin M35 for inactivation of Listeria monocytogenes in cold-smoked wild salmon. *Food Microbiology*, 26: 783-793.

Tyagi, A. K., Malik, A., 2011, Antimicrobial potential and chemical composition of Eucalyptus globulus oil in liquid and vapour phase against food spoilage microorganisms. *Food Chemistry*, 126: 228 Che.

Van Tassell, J.A., Martin, N.H., Murphy, S.C., Wiedmann, M., Boor, K.J. and Ivy, R.A., 2012, Evaluation of various selective media for the detection of Pseudomonas species in pasteurized milk. *Journal of Dairy Science*, 95: 1568–1574.

Vargas, M., Albors, A., Chiralt, A., & González-Martínez, C., 2011. Water interactions and microstructure of chitosan-methylcellulose composite films as affected by ionic concentration. *LWT - Food Science and Technology*, 44: 2290-2295.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J., & Perez-Alvarez, J. A., 2007, Antifungal activities of thyme, clove and oregano essential oils. . *Journal of Food Safety*, 27: 91–101.

Zheng, L. Y., & Zhu, J. F., 2003, Study on antimicrobial activity of chitosan with different molecular weight. *Carbohydrate Polymers*, 54: 527–530.

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 15، شماره 3، مرداد - شهریور 1398، ص. 27-13

Iranian Food Science and Technology Research Journal
Vol. 15, No. 3, Aug. Sep. 2019, p. 13-27

تهیه پوشش زیستی ضدسودوموناس با قرار دادن *لاکتوباسیلوس کازیی* ATCC 39392 در ماتریکس‌های سدیم کازئینات و متیل سلولز

هدی قیومی[1]- محمد علی نجفی[1*]- ناصر سلطانی[1]- محمد رهنما[2]

تاریخ دریافت: 1396/10/03
تاریخ پذیرش: 1397/08/28

چکیده

لفاف‌های زیست تخریب‌پذیر حاوی باکتری‌های اسید لاکتیک از روش‌های جدید نگهداری مواد غذایی است. در این مطالعه باکتری *Lactobacillus casei* ATCC 39392 (*L. casei* 39392) مستقیما به محلول سازنده لفاف‌های کازئینات سدیم و متیل سلولز به‌صورت جداگانه افزوده گردید، به‌طوریکه لفاف زیستی تهیه شده حاوی 10^6CFU/cm^{2}بود. خصوصیات مقدار رطوبت، حلالیت در آب، قابلیت نفوذ به بخار آب، رنگ، شفافیت، مقاومت در برابر کشش‌پذیری، ازدیاد طول فیلم تا نقطه پاره شدن و مدول الاستیک فیلم‌ها بررسی گردید. همچنین نرخ زنده‌مانی *L. casei* 39392 در طی 30 روز نگهداری (دمای 5 درجه سانتی‌گراد، رطوبت نسبی 75%) و اثر مهارکنندگی لفاف‌ها بر رشد باکتری *Pseudomonas aeruginosa* PTCC 10832 (*P. aeruginosa* 10832) طی 12 روز در 5 درجه سانتی‌گراد بررسی گردید. نتایج نشان داد افزودن *L. casei* 39392 به لفاف‌های تهیه شده شفافیت و نفوذپذیری به بخار آب بالاتری را در مقایسه با لفاف خالص نشان داد ($p<0/05$)، نرخ زنده‌مانی *L.casei* 39392 در لفاف سدیم کازئینات نسبت به لفاف متیل سلولز بالاتر بود ($p<0/05$)، بیشترین نرخ بازدارندگی رشد در برابر *P. aeruginosa* 10832 (85/3%) توسط لفاف زیستی متیل سلولز در روز چهارم نگهداری مشاهده گردید ($p<0/05$). نتایج ما نشان داد لفاف‌های زیستی حاوی *L.casei* 39392، می‌تواند تهیه لفاف‌های حاوی نگهدارنده‌های طبیعی را توسعه دهد.

واژه‌های کلیدی: آنتی-سودوموناس، فیلم خوراکی، *Lactobacillus casei*، متیل سلولز، سدیم کازئینات

1- گروه علوم و صنایع غذایی، دانشگاه زابل.
2- گروه بهداشت و کنترل کیفیت مواد غذایی، دانشگاه زابل.
(*- نویسنده مسئول : Email: najafi413@yahoo.com)