

Optimization of Nisin and EDTA Concentration in Antimicrobial Film Based on Psyllium Seed Hydrocolloid by Response Surface Methodology

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Abstract

Nowadays, due to the environmental problems caused by packaging based on synthetic polymers, the use of producing edible films based on biopolymers like polysaccharides, proteins, and fats has been interested. According to all the useful features of psyllium seed hydrocolloid (PH), the aim of this study was to evaluate the possibility of antimicrobial biodegradable edible film production using PH, glycerol, nisin (0, 5000 and 10000 IU/g) and EDTA (0, 15 and 30 wt%). The diameter of the inhibition zones against pathogenic bacteria on medium surface were measured as antimicrobial activity of films. The results showed that films containing nisin and EDTA significantly inhibition of *Staphylococcus aureus* and *Salmonella typhimurium* growth. With increasing concentration of nisin and EDTA the inhibitory power of film against pathogenic bacteria increased. Maximum inhibition was obtained at 3416.03 IU/g concentration of nisin and highest concentration of EDTA (30%). At the optimal point, the inhibition zone against *S. aureus* and *S. typhimurium* was 2.02 ± 0.2 and 2.2 ± 0.3 cm, respectively. Tensile and elongation of optimal film was 13.41 Mpa and 28.51%, respectively. According to results of this study, novel biodegradable antimicrobial PH film could be suggested as meat product packaging.

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Keywords

EDTA

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Introduction

Due to the increasing world population and the reduction of food resources, the preservation of food has been given a lot of attention. One of the main ways to deal with this issue is packaging of food (Mariniello et al., 2003). Nowadays, synthetic polymers are widely used in food packaging, but most of these materials increase environmental pollution due to their non-biodegradability

(Pérez et al., 2009) and some of them may produce compounds that are carcinogenic to humans. Biopolymers are biodegradable and eco-friendly materials, so the chemical risks caused by their consumption are reduced (Arvanitoyannis, 1999; Avila-Sosa et al., 2010). Recently, many researchers have increased their attention on the production of edible films based on biopolymers such as polysaccharides,

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proteins and lipids (Andreuccetti et al., 2011; Ghanbarzadeh, Almasi, & Entezami, 2011). Many studies have been conducted to use these biopolymers with other compounds such as plasticizers, antimicrobials and/or bioactive compounds to improve the mechanical, physical, functional, nutritional and organoleptic properties of edible films (Gaudin et al., 1999; Myllärinen, Partanen, Seppälä, & Forssell, 2002; Váscenez et al., 2009). Plasticizers provide more flexibility in the packaging film by reducing the intermolecular interactions and are likely to cause significant changes in the barrier properties of the final film (García, Martino, & Zaritzky, 2000). Studies show that not all edible films are good barriers against gases and water vapor, but they can carry antimicrobial agents and active compounds and help preserve food. A lot of research has been done on the use of edible films as carriers of antimicrobial compounds in food products and also to release antimicrobial substances into food products (Ahmadi et al., 2012).

Psyllium is a plant from the Plantago family. This plant is annual and about 200 species of this genus are widely distributed in different regions of the world (Ahmadi et al., 2012). *Plantago ovata* Forsk is a species of *Plantago* that is widely grown in India and also in Iran. Common names of *P. ovata* Forsk are Indian Plantago, Blond psyllium Spagel and Ispaghula (meaning horse ear in Hindi) (Ahmadi et al., 2012). Psyllium seed shell (*P. ovata* Forsk) as a source of psyllium hydrocolloid (PH) is widely used in various industries, especially pharmaceutical and food industries (Ahmadi et al., 2012). In the food industry, psyllium hydrocolloid is used to enhancing the consistency and stability and as gel constituting (Ahmadi et al., 2012).

Recently, attention has been paid to the use of food materials with antimicrobial packaging properties because they are very effective in preventing spoilage or killing pathogenic microorganisms that contaminate food. In addition, in this

method, unlike the method of direct use of additives in food, the development of unpleasant taste in food is limited. Therefore, these packaging materials can be used to prevent microbiological spoilage of perishable food products and maintain the quality and increase the shelf life of food (Bie et al., 2013). Transferring antimicrobial compounds to reduce undesirable bacteria in food through packaging film has been done for years. Various perspectives have been proposed for the use of edible and polymeric films to deliver bacteriocins to different food surfaces (Cutter, Willett, & Siragusa, 2001).

Nisin is a natural antibacterial compound produced by *Lactococcus lactis* and is able to effectively inhibit the growth of a wide range of Gram-positive microorganisms and prevent spore germination (Yang, Lin, Sung, & Fang, 2014). It is the first antimicrobial peptide approved in the United States for use in processed cheese with "generally recognized as safe" status. Also, the use of nisin in various food products is allowed in several countries (Sanjurjo, Flores, Gerschenson, & Rosa, 2006). Researches has shown that nisin affects gram-positive [G(+)] bacteria better than gram-negative [G(-)] bacteria. The reason for this is that the cell wall of gram-positive bacteria has a large negative charge, and therefore an electrostatic interaction between the positive charges in nisin and the negatively charged cell surface is created, and nisin can attach itself to the bacterial cell membrane (Cole & Nizet, 2016), The hydrophobic amino acid of the nisin peptide enters the bacterial cell wall and changes the permeability of the bacterial cell membrane, and this causes the death of the bacteria (Lei et al., 2019).

Ethylenediaminetetraacetic acid (EDTA) dissociates divalent cations (especially Mg^{2+} and Ca^{2+}) that contribute to the stability of the outer membrane of gram-negative bacteria (G. J. Tsai & Su, 1999). Therefore, EDTA will improve the

activity of nisin against Gram-negative bacteria (G. U. O. Tsai, Su, Chen, & Pan, 2002). The use of EDTA in foods and food supplements was approved in the European Union (EU) (EFSA 2010; EU 2010; EU 2011) after earlier food approvals in China (PRC 1994; PRC 1996), the Philippines (Republic of the Philippines 1995), most Latin American countries by about 2000, United States (FDA 2004; FDA 2006), Australia and New Zealand (FSANZ 2008), and India (Gazette of India 2011).

Nisin is incorporated into various polymers. For example low-density polyethylene (LDPE) film containing nisin to protect cattle carcasses from *B. thermosphacta* contamination was developed (Siragusa, Cutter, & Willett, 1999). Pediocin or nisin, with or without a chelating agent was incorporated into a food packaging film and then it used to protect food against harmful bacteria (Ahmadi *et al.*, 2012; Lambert, Smith, & Dodds, 1991). The objective of this research was to study the effect of edible films based on psyllium seed hydrocolloid (PH) containing nisin & EDTA on *Staphylococcus aureus* and *Salmonella typhimurium* growth.

Materials and methods

Materials

The psyllium seeds were procured from the local medical market in Mashhad and ethanol (96%) was obtained from Taghtirkhorasan (Mashhad, Iran), respectively. *Staphylococcus aureus* PTCC 1764 and *Salmonella typhimurium* PTCC 1609 prepared from research Institute of Food Science and Technology (Mashhad, Iran). Brain Heart Infusion (BHI) agar and was purchased from Merck (Darmstadt, Germany).

Extraction of psyllium hydrocolloid

About 10 g psyllium seeds was sieved and washed for three times with ethanol with triple of its weight for 15 min under constant stirring and then dust, stone and chaff removed from it by filtration

(Ahmadi *et al.*, 2012). The seeds dried in an oven at 70 °C for an hour and then cleaned seeds were dispersed in 120 mL distilled water at 70 ± 10 °C for 90 min under stirring (1200 rpm) (IKA RTC basic, Germany). To make up the water loss as a result of evaporation, water (70 °C) was added at different intervals to the system. The hydrocolloid Extracted by filtration and then the filtrate dried at 40 °C overnight in a laboratory oven and dried PH was milled with a laboratory mill (IKA A11 basic, Germany).

Preparation of antimicrobial edible film

To prepare the film solutions, 1.8 g of PH resin powder was added and mixed well with 150 ml of de-ionized water (DI water). Glycerol at a concentration of 30% (w/w) of PH was added and stirred by a magnetic stirrer (1200 rpm) at 70 ± 10 °C for 1 h. After that nisin at three levels: (0, 5000, 10000 IU/g) and EDTA at three levels: (0, 15, 30 wt% of the PH) was added to the film-forming solution under constant stirring (1200 rpm) at 70 °C for 30 min. Then a vacuum-oven applied for 15 min at 70 °C to remove air bubbles. The prepared solution was casted onto a Teflon plate and dried in oven at 30 ± 10 °C for 48 h. The obtained film was peeled from the plate and conditioned at 23 ± 2 °C in a desiccator containing saturated solution of $\text{Ca}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ($50 \pm 2\%$ relative humidity, RH) for at least 48 h prior to tests.

Antimicrobial activity

Antimicrobial activity assay performed based on measurement of growth inhibition zone diameter by a micrometers (T F, 1/50 mm, Germany). Prior to inoculum preparation, *S. aureus* and *Salmonella typhimurium* were grown in tryptic soy broth (TSB: Remel Inc., Lenexa, Kans., U.S.A.), and *S. aureus* was grown aerobically at 35 °C for 18 h in BHI broth (Millette, Le Tien, Smoragiewicz, & Lacroix, 2007). Nisin, and EDTA, incorporated into PH films were evaluated for antimicrobial activities against *S.*

Aureus and *Salmonella typhimurium*. One of the key principles in antimicrobial film production is release of antimicrobial compounds. Bacterial growth inhibition by antimicrobial films was evaluated using an agar diffusion method and a liquid incubation method (Appendini & Hotchkiss, 2002).

In the agar diffusion test, each film sample (5 mm diameter) was placed on the surface of a BHI agar plate overlaid with the seeded semi-soft BHI agar (0.5% (w/v) agar). The seed density of overlay was approximately 10^6 CFU/mL of *S. aureus* or *S. typhimurium*. The agar plates were incubated at 37 °C for 24 h. Diameters of inhibition zone around film specimen were used to determine antimicrobial activity of each film sample. (Jin & Zhang, 2008). Finally, physical and mechanical properties of optimized film were evaluated.

Physicomechanical Properties of film

Thickness of film

The thickness was measured by a micrometer with an accuracy of 0.001 mm at at least 10 random positions of the optimal film sample (Mitutoyo Corp. MDC-1 SB, Japan) and the average thickness was reported.

Water vapor permeability

A modified ASTM E96 method was used to measure the water vapor permeability (WVP) of the optimized film sample (ASTM, 1995). The film was first sealed with paraffin on a glass cell (0.01178 m² of film surface) containing some anhydrous calcium chloride desiccant. The cell was placed inside a desiccator containing a saturated sodium chloride solution at a test temperature of 25°C. Cells were then weighed at 12-hour intervals over a 7-day period, and changes in cell weight were plotted as a function of time. The slope of the plot ($\frac{\Delta w}{\Delta t}$) was calculated and WVP of film was obtained from equation 1.

$$WVP = \frac{\Delta w \times x}{\Delta t \times A \times \Delta P} \quad (1)$$

Where X = film thickness (m), A = area of the exposed film surface (m²) and ΔP = difference of partial pressure (Pa). The test was conducted with three replicates.

Mechanical properties

Texture analyzer unit (model TA-XT-Plus, U.K) was used to check the tensile properties of the film. For this purpose, the ASTM D882 standard test method was used on the optimal film sample (ASTM, 2002). The film was cut in dimensions of 1 x 6 cm. The distance between the two jaws of the machine was set at 4 cm and the speed of the upper jaw was set at 50 mm/min (Khoshgozaran-Abras, Azizi, Hamidy, & Bagheripoor-Fallah, 2012). Factors such as tensile strength (TS) and elongation at break (EB) were obtained from the stress-strain curve. This test was performed with three repetitions and the average values of TS and EB were reported

Statistical analysis

Central composite design (CCD) was used to investigate the effect of independent variables on dependent variables. The type of CCD used in this study was a face-centered composite (CCF) experimental design to determine the optimal levels of nisin and EDTA in antimicrobial films. In this case, the independent variables are investigated at 3 levels (-1, 0 and +1). The independent variables included the concentration of nisin and EDTA and the dependent variables were the growth inhibition zone diameter of bacteria. Then analysis of variance (ANOVA) was performed with a confidence level of 95% for each response variable in order to test the significance and fit of the model. Microsoft Windows Excel 2013 and Design-Expert (version 7.0.0) were used for data analysis.

Results and discussion

Investigation of the effect of EDTA and Nisin concentration on responses

The values of independent variables determined by CCD and the responses obtained in the multivariate study for each experiment are shown in Table 1. As it can

be seen the experiments contain a total of 13 different combinations, including five replicates of centre point.

Table 1. Central composite design of two variables with their observed responses.

| Number | Nisin (IU/g) | EDTA (wt%) | inhibition zone diameter (mm) | |
|--------|--------------|------------|---|--|
| | | | <i>S. aureus</i> , 10 ⁶ (cfu/ml) | <i>S. typhimurium</i> , 10 ⁶ (cfu/ml) |
| 1 | 0 | 30 | 1.9 | 2.8 |
| 2 | 5000 | 15 | 1.6 | 1.4 |
| 3 | 5000 | 15 | 1.8 | 1.3 |
| 4 | 5000 | 15 | 1.7 | 1.3 |
| 5 | 5000 | 15 | 1.8 | 1.4 |
| 6 | 5000 | 0 | 1.2 | 0.9 |
| 7 | 0 | 0 | 0 | 0 |
| 8 | 10000 | 0 | 1.4 | 0.9 |
| 9 | 10000 | 15 | 1.7 | 1 |
| 10 | 5000 | 15 | 1.6 | 1.3 |
| 11 | 10000 | 30 | 2.4 | 1.4 |
| 12 | 5000 | 30 | 2 | 2.4 |
| 13 | 0 | 15 | 1.1 | 1.4 |

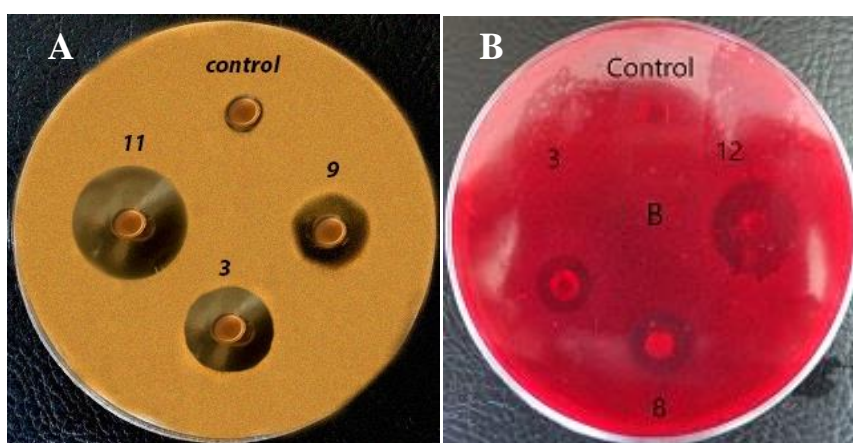


Fig. 1. Antimicrobial activity of films against *S. aureus* and *S. typhimurium* by disk diffusion method. *S. aureus*(A) and *S. typhimurium* (B).

As it was said before in this study antimicrobial activity of the films against bacteria expressed in terms of growth inhibition zone diameter (Fig. 1).

Among the different regression models proposed by RSM, quadratic model was selected for predicting the behavior of each of responses as affected by independent variables variations. Model significance, coefficient of determination (R^2) and Lack of Fit significance were considered for evaluating the suitability of models with R^2 as a measure of the model's overall performance.

Antimicrobial activity of films against *S. aureus*

An ANOVA table is commonly used to summarize the tests performed. The ANOVA table for response surface quadratic model for the predicting of antimicrobial activity of films against *Staphylococcus aureus* was given in Table 2. The value of p (0.0006) less than 0.05 indicates that the obtained model is statistically significant. High F value (19.22) indicates that the variation of the nisin and EDTA concentration makes a big change on the antimicrobial activity of film against *Staphylococcus aureus*. ANOVA

for the model gave a coefficient of determination of 97.9%, which indicates a close agreement between experimental and predictive values. Finally the Lack of fit of model (0.1281) is no significant, so considering all of these statistics, one can conclude that this model is quite suitable for prediction of antimicrobial activity of the films in terms of concentration of nisin and EDTA in PH films. The second-order polynomial equation obtained is as equation 2:

$$Y = 0.14511 + 2.4195E-004A + 0.061398B - 3E-006AB - 9.5862E-009A^2 - 1.76245E-004B^2 \quad (2)$$

Where Y is growth inhibition zone diameter for 10^6 (cfu/ml) dilution of *Staphylococcus aureus*. A and B are nisin and EDTA concentrations respectively, AB is the linear interaction of nisin and EDTA, and A^2 and B^2 are the quadratic effect of nisin and EDTA.

As seen in Table 2 nisin and EDTA concentration, nisin square and the interaction between nisin and EDTA are the significant parameters and only the EDTA square has no significant effect on the antimicrobial activity of the films against *S. aureus*.

Table 2. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for the *Staphylococcus aureus*

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob > F | |
|----------------|----------------|----|-------------|---------|------------------|-----------------|
| Model | 4.59 | 5 | 0.92 | 19.22 | 0.0006 | significant |
| A-Nisin | 0.88 | 1 | 0.88 | 18.45 | 0.0036 | |
| B-EDTA | 3.08 | 1 | 3.08 | 64.49 | < 0.0001 | |
| AB | 0.3 | 1 | 0.3 | 6.33 | 0.04 | |
| A ² | 0.33 | 1 | 0.33 | 6.8 | 0.035 | |
| B ² | 0.068 | 1 | 0.068 | 1.42 | 0.2718 | |
| Residual | 0.33 | 7 | 0.048 | | | |
| Lack of Fit | 0.24 | 3 | 0.081 | 3.51 | 0.1281 | not significant |
| Pure Error | 0.092 | 4 | 0.023 | | | |

The response surface plot (Fig. 2) indicates that with increasing the nisin and EDTA concentration in PH films, antimicrobial activity against *S. aureus* increased. The minimum antimicrobial activity is observed for control (film without nisin and EDTA). Chang et al.

(2021) studied the antibacterial activity of chitosan-poly(lactic acid) (PLA) composite film combined with nisin and EDTA. They reported that the addition of EDTA + nisin to the chitosan-PLA matrix significantly improved the antibacterial activity of the blend film.

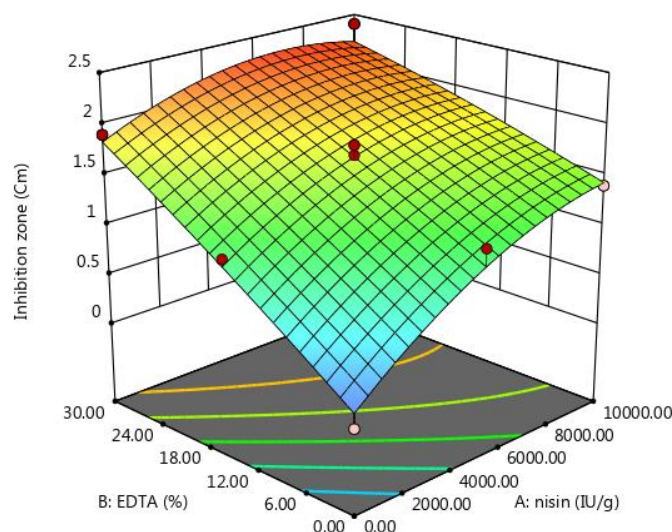


Fig. 2. Response surface plot showing the effect of nisin and EDTA concentration in PH films on growth inhibition zone diameter against *Staphylococcus aureus* (10^6 cfu/ml).

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for the *Salmonella typhimurium*

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob > F | |
|----------------|----------------|----|-------------|---------|------------------|-----------------|
| Model | 6.36 | 5 | 1.27 | 77.89 | < 0.0001 | significant |
| A-Nisin | 0.027 | 1 | 0.027 | 1.63 | 0.2419 | |
| B-EDTA | 4.17 | 1 | 4.17 | 255.28 | < 0.0001 | |
| AB | 1.44 | 1 | 1.44 | 88.23 | < 0.0001 | |
| A ² | 0.68 | 1 | 0.68 | 41.72 | 0.0003 | |
| B ² | 0.25 | 1 | 0.25 | 15.58 | 0.0056 | |
| Residual | 0.11 | 7 | 0.016 | | | |
| Lack of Fit | 0.094 | 3 | 0.031 | 6.28 | 0.054 | not significant |
| Pure Error | 0.02 | 4 | 5.00E-03 | | | |

Antimicrobial activity of films against *S. typhimurium*

The ANOVA table for response surface quadratic model for the predicting of antimicrobial activity of films against *Salmonella typhimurium* was given in Table 3. The value of p (< 0.0001) less than 0.05 indicates that the obtained model is statistically significant. The high F value (77.89) indicates the high impact of the independent variable on the dependent variable. Therefore, here the high F (77.89) shows that changing the concentration of nisin and EDTA makes a big change in the antimicrobial activity of the film. ANOVA for the model shows a coefficient of determination of 98.2%, which indicates the high ability of predicting the experimental values by the model. Finally

the Lack of fit of model (0.054) is not significant, so considering all of these statistics, one can conclude that this model is quite suitable for prediction of antimicrobial activity of the films in terms of concentration of nisin and EDTA in PH films. The second-order polynomial equation obtained is as equation 3:

$$Y_2 = 0.090517 + 1.86379E-004A + 0.065460B - 7.66667E-006AB - 1.01379E-008A^2 - 8.73563E-004B^2 \quad (3)$$

Where Y is growth inhibition zone diameter for 10^6 (cfu/ml) dilution of *S. typhimurium*. A and B are nisin and EDTA concentrations respectively, AB is the linear interaction of nisin and EDTA, and A

A^2 and B^2 are the quadratic effect of nisin and EDTA.

As seen in Table 3, EDTA concentration, nisin square, EDTA square and the interaction between nisin and EDTA are the significant parameters and only the nisin concentration has no significant effect on the antimicrobial activity of the films against *S. typhimurium*.

The response surface plot (Fig. 3) indicates that with increasing the EDTA concentration in PH films, antimicrobial activity against *S. typhimurium* increased. Also, with increasing nisin concentration at low concentration of EDTA, antimicrobial activity against *S. typhimurium* increased but at high concentration of EDTA (30%) with increasing nisin concentration, antimicrobial activity against *S. typhimurium* decreased. It seems that nisin has an antagonistic effect (on antimicrobial activity against *Salmonella typhimurium*)

in high concentrations of EDTA. The minimum antimicrobial activity is observed for control (film without nisin and EDTA). soy protein based edible film containing 1% grape seed extract, 10,000 IU/g, nisin and 0.16% EDTA was produced and reported that it was able to reduce the population of *e. Listeria monocytogenes* by 2.9 logCFU/ml, while the population of *Escherichia coli* O157:H7 and *S. typhimurium* were reduced by 1.8 and 0.6 logCFU/ml, respectively (Sivaroban, Hettiarachchy, & Johnson, 2008). Also, it was showed that polyvinyl chloride film containing nisin can reduce the population of *Salmonella typhimurium* in the skin of inoculated broilers by 4.3 log (Natrajan & Sheldon, 2000).

Nisin is able to form pores in the cell membrane, and loss of proton motive force is one of the proposed mechanisms of Nisin's activity (McMahon, 2002).

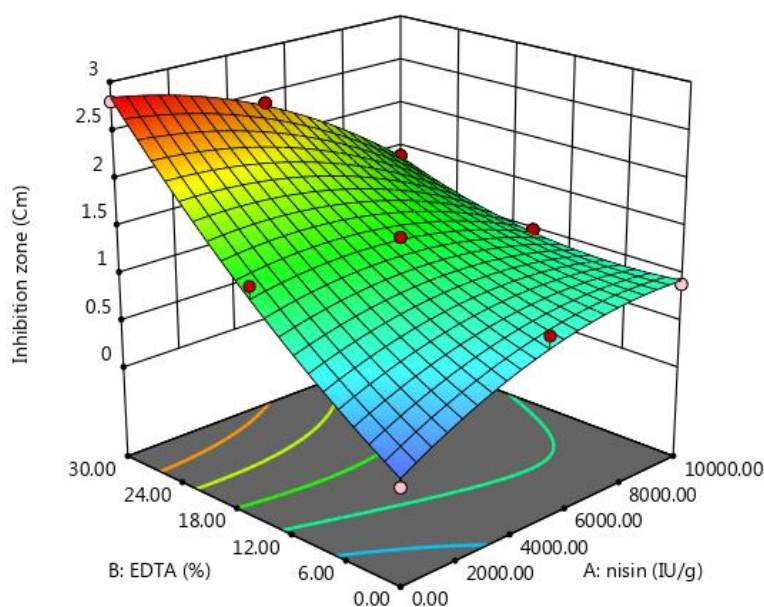


Fig. 3. Response surface plot showing the effect of nisin and EDTA concentration in PH films on growth inhibition zone diameter against *S. typhimurium* (10^6 cfu/ml).

Analysis of optimization and model validation

Optimal values of nisin and EDTA concentration in PH films for maximum inhibition of *S. aureus* and *S. typhimurium* growth, obtained as 3416.03 IU/g nisin and

30 wt% EDTA by response surface methodology (RSM). Also the optimal diameter of the inhibition zone for *S. aureus* and *S. typhimurium* was predicted as 2.18 cm and 2.57 cm, respectively. To confirm the model validation, an experiment with

three replicates at optimal conditions (3416.03 IU/g nisin and 30% EDTA) was done. The Experimental and Predicted diameter of the inhibition zone for *S. aureus*

and *S. typhimurium* are shown in Table 4, where absence of significant difference between the data confirmed the efficiency of the model.

Table 4. The Experimental and Predicted diameter of the inhibition zone for *S.aureus* and *S. typhimurium*

| bacteria | Dilution (CFU/ml) | Inhibition zone (cm) | |
|-----------------------|-------------------|------------------------|-------------------|
| | | Experimental | Predicted |
| <i>S. aureus</i> | 10 ⁶ | 2.0 ± 0.2 ^A | 2.18 ^A |
| <i>S. typhimurium</i> | 10 ⁶ | 2.2 ± 0.3 ^B | 2.57 ^B |

Physical and tensile properties of optimal film

The physical and tensile properties of the optimal film were measured with three replicates. The obtained film was slightly hued but still homogeneous, flexible and transparent (Fig. 4).

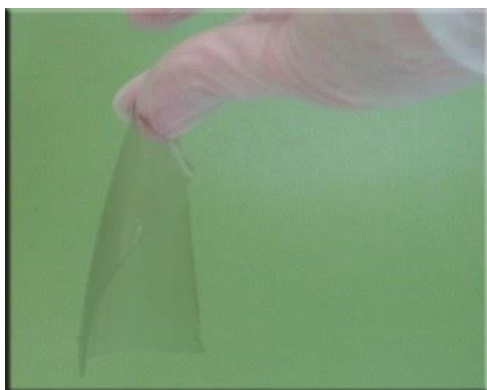


Fig. 4. Psyllium seed hydrocolloid (PH) film (30% (w/w) glycerol, 3416.03 IU/g nisin and 30% EDTA).

The average thickness of optimal film was 0.7 mm. In previous study the films produced from psyllium seed hydrocolloid with different concentrations of glycerol had different thicknesses varied between 0.050 and 0.72 mm depending on the concentration of glycerol used (Ahmadi *et al.*, 2012). Water vapor permeability (WVP) is one of the most important properties of films because it is closely related to deterioration reactions (Ahmadi *et al.*, 2012). The presence or absence of holes or gaps in the structure of biopolymers and their hydrophilic or hydrophobic nature greatly affects the WVP of films (Vásconez *et al.*, 2009). The WVP of the optimal film was $1.31 \pm 0.02 \times 10^{-10} \text{ g m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$. Similar results were

obtained by other researchers and the observed difference in results may be due to differences in the percentage of glycerol, thickness of film and the presence of nisin and EDTA in the formulation of the film (Ahmadi *et al.*, 2012). Studies show that PH film has higher WVP than corn bran arabinoxylan films (Péroval, Debeaufort, Despré, & Voilley, 2002). These differences are probably due to the difference in the laboratory conditions, the difference in the thickness of the PH layers, or the source of arabinoxylan of the prepared films.

Percentage of elongation at break (EB) and tensile strength (TS) are two important properties for packaging material. The results showed that TS and EB of the optimal film was 13.41 Mpa and 28.51%, respectively. Films from psyllium seed hydrocolloid were produced and it was reported that by increasing of glycerol concentration, the TS of PH film decreased and EB of PH film increased significantly (Ahmadi *et al.*, 2012). These researches concluded that increasing the concentration of plasticizer (glycerol) in the PH film improves the elasticity of the film and reduces its resistance. The TS of PH film was lower than corn bran arabinoxylan films with glycerol as plasticizer. While the EB of PH films was higher than the EB of corn bran arabinoxylan films (Péroval *et al.*, 2002). These observed differences are probably related to the difference in the structure of arabinoxylan.

Conclusions

One of the most important bacteria caused food spoilage, especially for meat products

is happened by *Staphylococcus aureus* (as a Gram-positive bacteria) and *Salmonella typhimurium* (as a Gram-negative bacteria). The use of natural polymer films containing nisin and EDTA for the control of pathogens in food could reduce the incidence of foodborne disease and could control the quality of the food products. This study showed psyllium hydrocolloid containing antimicrobial agents (nisin and EDTA) can be used as an antimicrobial coating on the surface of solid foods or packaging of these. The results of this study show that incorporation of natural antimicrobial substances such as nisin and EDTA to psyllium seed hydrocolloid film can provide a system for improving the effectiveness of bacteriocins in food products. In vitro bacteriocin activity assay showed that release of nisin and EDTA

from the film is done well and and it could considerably prevent the growth of bacteria. Optimal values of nisin and EDTA concentration in PH films for maximum inhibition of *S. aureus* and *S.typhimurium* growth, obtained as 3416.03 IU/g nisin and 30 wt% EDTA by response surface methodology (RSM). Also the optimal diameter of the inhibition zone for *S. aureus* and *S. typhimurium* was predicted as 2.18 cm and 2.57 cm, respectively. The average thickness of optimal film was 0.7 mm. The WVP of the optimal film was $1.31 \pm 0.02 \times 10^{-10} \text{ g m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ and the results showed that TS and EB of the optimal film was 13.41 Mpa and 28.51%, respectively. This is a pilot study, and it is a starting point to determine whether psyllium seed hydrocolloid /nisin/ EDTA film has the potential for antimicrobial packaging.

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بهینه‌سازی غلظت نایسین و EDTA در فیلم ضد میکروبی حاصل از هیدروکلوئید دانه اسفرزه با روش سطح پاسخ

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چکیده

امروزه با توجه به مشکلات زیست محیطی ناشی از بسته بندی براساس پلیمرهای مصنوعی، استفاده از تولید فیلم‌های خوراکی بر پایه بیوپلیمرهایی مانند پلی ساکاریدها، پروتئین‌ها و چربی‌ها مورد توجه قرار گرفته است. با توجه به تمام ویژگی‌های مفید هیدروکلوئید دانه اسفرزه (PH)، هدف این مطالعه بررسی امکان تولید فیلم خوراکی زیست تخریب پذیر ضد میکروبی با استفاده از PH، گلیسرول (به عنوان نرم کننده)، نایسین و EDTA (به عنوان ضد میکروب) بود. فیلم‌ها با سطوح مختلف نایسین (۰، ۵۰۰ و ۱۰۰۰ IU/g) و EDTA (۰، ۱۵ و ۳۰ درصد وزنی) تهیه شدند. قطر نواحی بازدارنده رشد باکتری‌ها به عنوان معیاری برای فعالیت ضد میکروبی فیلم در نظر گرفته شد. نتایج نشان داد که فیلم‌های حاوی نایسین و EDTA به طور قابل توجهی از فعالیت استافیلوکوکوس اورئوس (به عنوان یک باکتری گرم مثبت) و سالمونلا تیفی موریوم (به عنوان یک باکتری گرم منفی) جلوگیری می‌کنند. با افزایش غلظت نایسین و EDTA قدرت بازدارندگی فیلم در برابر رشد باکتری افزایش یافت. حداکثر مهار در غلظت ۳۴۱۶/۰۳ IU/g نایسین و بالاترین غلظت EDTA (۳۰ درصد) مشاهده شد. در نقطه بهینه منطقه بازدارنده از رشد برای استافیلوکوکوس اورئوس و سالمونلا تیفی موریوم به ترتیب برابر ۲/۰۲±۰/۲ و ۲/۲±۰/۳ بود. خواص فیزیکی و مکانیکی فیلم بهینه اندازه گیری و گزارش شد. با توجه به نتایج این مطالعه، این فیلم‌های ضد میکروبی زیست تخریب پذیر جدید می‌توانند به عنوان بسته بندی محصولات گوشتی پیشنهاد شوند.

واژه‌های کلیدی: EDTA، نایسین، هیدروکلوئید دانه اسفرزه، فیلم خوراکی