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Application of zein antimicrobial edible film incorporating *Zataria multiflora* boiss essential oil for preservation of Iranian ultrafiltered Feta cheese

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Zein based edible film was developed and incorporated with *Zataria multiflora* boiss essential oil. Mechanical and microbiological characteristics of this biofilms were measured. Increasing concentration of antimicrobial agent in film reduced stretchability, tensile strength and elongation, however increased the thickness and water vapor transmission parameters (P<0.05). Addition of *Z. multiflora* boiss essential oil, resulted in decreasing the count of viable *Salmonella enteritidis, Listeria monocytogenes, Escherichia coli* and *Staphylococcus aureus* in the produced cheese. This investigation concludes that the biofilm containing *Z. multiflora* boiss essential oil can be highly recommended for packaging of Feta cheese with the improved microbiological and sensory quality. Besides, application of natural plant essential oil has economical and health promoting benefits.

Key words: Zein, *Zataria multiflora*, cheese, pathogens, mechanical properties.

INTRODUCTION

Dairy products are nutritionally valuable parts of the human diet, the demand for which is set to grow, globally. Shelf life as an important element influencing the quality of dairy products may be shortened by microbial growth. It can be extended by applying thermal treatment during manufacture and addition of preservatives. The combination of the increasing occurrence of food-borne disease and the results of social and economic problems indicate there is a steady demand to produce new antimicrobial agents (Palmer et al., 2001). Concerns for the safety of some chemical preservatives and negative customer feedbacks over their use have encouraged increasing concentration in more natural green alternatives for the extension of product shelf-life. The antimicrobial agents might be added directly to the product formulation to reduce the growth or survival of food-borne bacteria.

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Essential oils have been known for their biological activities for many decades and they should, in principle, not be toxic to human and could replace toxic synthetic fungicides. However, the food industries have been encouraged for more application of antimicrobial agents (Arora and Kaur, 1999; Appendini and Hotchkiss, 2002; Naser-Abbas and Kadir-Halkman, 2004). The ingredients of edible films are important to reduce the growth of microorganisms. The agents of edible films show an important role to the surface of the product (Ayana and Turhan, 2009).

In the past concern of food packaging was good looking, but today new consideration are of interests including environmental impact (Joerger, 2007), efficiency of heat and mass transfer, intelligent and active packaging. Active packaging is a new conception that leads to extend the shelf life of the product or improve sensory of the product (Devlieghere et al., 2004). Incorporating anti-microbial agents in food packages (antimicrobial active packaging) could be more efficient than the direct addition of these compounds into the food. Besides, migrations of the active compound from the packaging material into the food enable not only the inhibition of food-borne bacteria present in food, but also create a remaining activity over time, during transport, storage and distribution of food (Bolgar et al., 2007).

An edible film as a primary packaging made from edible components can be applied to improve sensory, shelf life, mass transfer and mechanical properties of various food products (Krochta, 2002; Janjarasskul and Krochta, 2010) as well as health benefits (including vitamins, minerals and bioflavonoids) (Larotonda et al., 2005; Park and Zhao, 2006; Park et al., 2011). The biodegradability of edible coatings is other desirable benefits associated with their use (Krochta, 2002; Siracusa et al., 2008). Edible polymeric packaging materials can be made from polysaccharides (for chitosan, carrageenan), proteins and lipids. The edible films which are usually between 50 to 250 µm in thickness can be used as wrapping materials, stand-alone films, or can be fabricated into pouches and bags for subsequent packaging use. Edible films are distinguished from coatings by their method of manufacture and application to the food product. Films are dried preformed thin material structures that are used on or between layers of food components (Pascall and Lin, 2013).

Zein is a natural protein found in corn seeds. It is a unique and complex material, and one of the few cereal proteins extracted in a relatively pure form (Panchapakesan et al et al., 2012). Zein based films could act as barriers to oxygen, carbon dioxide, and oils, thus helping the prevention of the deterioration of food quality and extension of the shelf-life of food products. Zein is not soluble in water but soluble in 40 to 90% ethanol. The water-insoluble characteristic of zein makes it a good candidate for the development of natural biopolymeric edible films (Shukla and Cheyran, 2001).

Several studies have focused on application of herbal extracts and essential oils including antimicrobial phenolic compounds in film packaging (Shaffiee and Javadian, 1997; Seydim and Sarikus, 2007). *Z. multiflora* boiss. is a plant that belongs to the Laminaceae family and grows only in Iran, Pakistan and Afghanistan (Ali et al., 2000). Among 215 species of *Z. multiflora* (known as Thymus and ‘‘Avishan” in Persian) grown in the world, 14 species are distributed in Iranian flora (Jalas, 1982). Leaves and flowering parts of this plant are well known as medicinal plants due to their health benefits as analgesic and carminative specifications, antiseptic and carminative as well as treating colds, tonic and herbal tea (Mozzaffarian, 1998). It is used as a traditional cure for its antiseptic, analgesic, carminative specifications (Mozzaffarian, 1998) in treatment of Rheumatism, and skin disorders. Also, this plant is applicable for preservation of several food products in Iran due to strong microbial inhibition and flavoring (Hosseinzadeh et al., 2000). Its antimicrobial activity is well established against a wide variety of bacteria (Aktug and Karapinar, 1986; Karaman et al., 2001; Palmer et al., 2001; Delgado et al., 2004; Fazeli et al., 2007; Altiok et al., 2012) and fungi (Rana et al., 1997; Karman et al., 2001), and attributes to the phenolic compounds such as carvacrol and thymol (Ali et al., 2000; Bagamboula et al., 2004).

Although, there are several reports on essential oil compositions of different Thymus species, investigations on their biological activities are still scarce. Nejad Ebrahimi et al. (2008) have reported the essential oil compositions of some Iranian Thymus species. The antibacterial and antifungal activity of *Thymus revolutus* oil from Turkey (Karaman et al., 2001), *Thymus pubescens* and *Thymus serpyllum* (Rasooli and Mirmostafa, 2002) as well as antioxidant activities of the oils of *Thymus caespititius, Thymus camphorates* and *Thymus mastichina* from Portugal have been reported (Miguel et al., 2004). Antimicrobial packaging decreases the growth of some microorganisms by using the slides of the films directly on some solid foods for example, cheese (Cagri et al., 2003).

Infected cheese can cause some diseases such as Gastroenteritis and Staphylococcal food poisoning that sourced by *Escherichia coli* and *Staphylococcus aureus* (Ayana and Turhan, 2009). According to Ghasemi et al. (2012) study, there was a decline in the growth of microorganisms by increased concentration of ZEO. *Salmonella enteritidis, Listeria monocytogenes, E. coli* and *S. aureus* showed significant reductions in bacterial survival in the higher ZEO concentration.

In this investigation, *Z. multiflora* was incorporated into zein based films aiming to control the growth of *S. enteritidis, L. monocytogenes, E. coli and S. aureus.* Furthermore, this work evaluated tensile strength and gas permeability characteristics of the films. The objective of this study was to investigate the role of packaging technology in extending the shelf life of cheese and reducing the risk of the pathogens.

Table 1. Composition table of *Zataria* essential oil.

Number	Composition	*Zataria multiflora*	
		Retention time (min)	Concentration (%)
1	α-Pinene	10.3	1.4
2	Camphene	10.8	-
3	β-Pinen	11.8	-
4	β-Myrcene	12.3	0.8
5	β-Phellandrene	12.8	-
6	carene	13.2	-
7	p-Cymene	0.513	8.5
8	γ-Terpinene	14.6	6
9	α-Terpineol	19.1	-
10	Thymol	22.5	48.2
11	Carvacrol	22.8	13.8
12	Caryophyllene	25.1	-
Total			78.7

MATERIALS AND METHODS

Z. multiflora boiss. essential oil preparation

Z. multiflora boiss. was purchased from Soha4 company (Tehran, Iran). 50 g dried leaves of plant was placed into a flask and the essential oil was extracted with a clevenger-type apparatus using hydro-distillation method for 2 h until no more essential oil was obtained (Didry et al., 1993). The essential oil was collected, dried with anhydrous sodium sulphate and stored at 4°C and kept for further usage. Table 1 shows the composition of ZEO used in this study.

Gas chromatography/mass spectrometry analysis

The GC–MS analyses were performed using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) equipped with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization 115 (70 eV). Oven temperature initially was kept at 50°C for 5 min and raised at the rate of 3°C/min to 240°C and reached to 290°C at 15°C/min then held isothermal for 3 min. Carrier gas was Helium at flow rate of 0.8 ml/min. 1 µl of samples and external standard were injected manually in the splitless mode. The components of the oil were identified based on the comparison of their retention time and with those of literature or with those of authentic compounds available in our laboratory and confirmed by matching their mass spectra with those of a computer library of the GC– MS data system and other published mass spectra. (C8–C22) n-alkanes were used as reference points in the calculation of relative retention indices (PRI) (Adams, 1995).

Inoculum preparation

L. monocytogenes ATCC 7644, *E. coli* 0157:H7 VT negative, *S. enteritidis* and *S. aureus* ATCC 25923 were obtained from Research Department of Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran. These were maintained on nutrient agar (Oxoid CM0003) slopes at 5°C. The above mentioned microorganisms were transferred from the cultures to the slopes and incubated for 24 h in 37°C. After that the procedure for the three sub-cultures was carried out to make sure the organisms were active and vital. The organisms were subcultured three times on consecutive days in nutrient broth (Oxoid CM0001) incubated at 37°C at precise 24 h intervals, followed by streaking on nutrient agar incubated at 37°C to check purity. The inoculum is prepared using the third sub cultured, which contained ca. 10^8 cfu/ml. Decimal dilutions are made to give a concentration of ca 10^6 cfu/ml. From this a volume of 500 µl was used to inoculate the broth combinations to give a final concentration of ca 10^4 cfu/ml (Ghasemi et al., 2012).

Preparation of films

The zein films are prepared according to casting method (Dry process). Zein (5.00 g) was dissolved in 45 ml of ethanol at 78°C. Glycerol was added (1.5 ml) as a plasticizer. Different concentration of *Z. multiflora* Boiss. (0, 1, 2, 3 and 4% w/v) were added. After degassing, the films were cast on glass plates and dried overnight at 22°C (Ayana and Turhan, 2009).

Packaging of surface-contaminated cheese with antimicrobial zein films

Cheese was bought from a local market, Tehran, Iran. Four microorganisms (two gram positive and two gram negative bacteria) of *S. enteritidis, L. monocytogenes, E. coli* and *S. aureus* were used. Cheese (100 g) inoculated separately with each bacterium. The slices of cheese were wrapped with zein control edible films and zein edible films containing 1, 2, 3 and 4% (w/v) of *Z. multiflora*. The slices of cheese were put into the refrigerator at 4°C for 14 days.

Film thickness

The thickness of the films was measured by a digital micrometer (Mitu-toyo, Mitutoyo Corporation, Japan) at five different positions and the mean of the thickness was used as a film thickness.

Water vapor permeability determination

The water vapor permeability is a measure for breathability or for an

Table 2. Mechanical properties of zein films formulated with *Zataria multiflora* Boiss essential oil.

Film formulation	Thickness (mm)	Tensile strength (MP$_a$)	Elongation at break (%)
Control	0.100	24.27	3.64
1% ZEO	0.100	6.98	1.33
2% ZEO	0.132	4.68	1.02
3% ZEO	0.195	2.04	0.86
4% ZEO	0.242	1.24	0.50

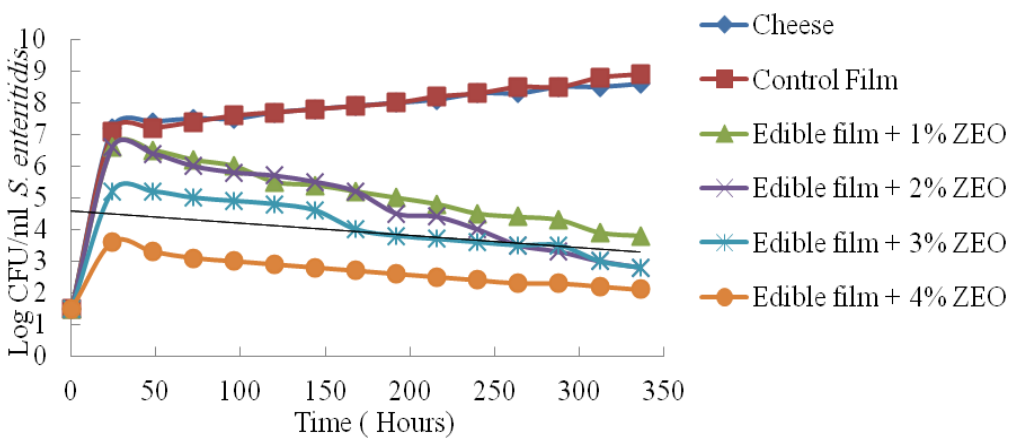

Figure 1. Inhibition of *Salmonella enteritidis* in cheese by zein edible film incorporated different concentrations of *Zataria multiflora* Boiss. essential oil.

ability of textile to transfer moisture (Hu et al., 2001). The water vapor permeability was measured according to ASTM E 96-95 (1995). A cup is filled with distilled water leaving a small gap (1.90 to 0.63 cm) of air space between the specimen and the water. The cup is then sealed to prevent vapor loss except through the test sample. An initial weight is taken of the apparatus and then periodically weighed over time until results become linear (Merck, Darmstadt, Germany). Caution must be used to assure that all weight loss is due to water vapor transmission through the specimen.

Mechanical properties of films

Mechanical characteristics were considered according to ASTM, 2001 method by using a Universal Testing Instrument Model AI-5000 (Gotech, Taiwan) fitted with a 50 N static load cell.

Statistical analysis

Each film attribute was measured in triplicate. The data were considered by SPSS software with (version 18.0, IBM, New York, NY) and Excel 2007 with a significance set at $P<0.05$.

RESULTS AND DISCUSSION

Mechanical properties

ZEO was added with different percentages to the Zein films to investigate the impact of oil concentration on mechanical properties of biofilm. As Table 2 shows, the thickness and mechanical properties of ZEO incorporated films will change through the incorporation of ZEO with zein films. The results show that the zein film without any combination is stronger and more stretchable than the film which was added ZEO. The films containing antimicrobial agents had thicknesses of 0.100 mm for 1% ZEO film and 0.242 mm for 4% ZEO film as evaluated with a thickness of 0.100 mm for control film. Also, the enhancement of antimicrobial agent leads to decreased tensile strength and elongation but increased films thickness ($P<0.05$).

Antimicrobial activity

Figures 1 to 4 show the effect of the zein films incorporated with 0, 1, 2, 3 and 4% (w/v) essential oil of *Z. multiflora* Boiss on the survival / growth of *S. enteritidis, E. coli, S. aureus* and *L. monocytogenes* over 14 days at 4°C. Results indicate a significant decrease was detected between the control films and different concentration of the essential oil on mentioned bacteria. In other words in presence of ZEO there is a sharp decrease of the bacteria in plates regarding the concentration of essential oil.

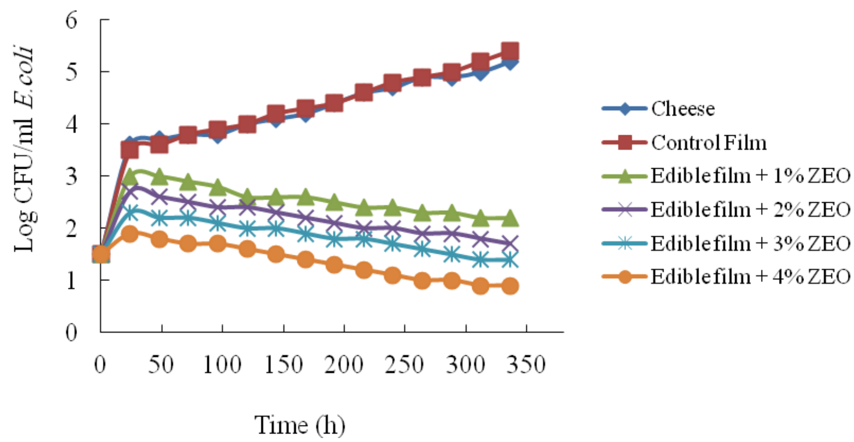

Figure 2. Inhibition of *Escherichia Coli* in cheese by zein edible film incorporated different concentrations of *Zataria multiflora* Boiss. essential oil.

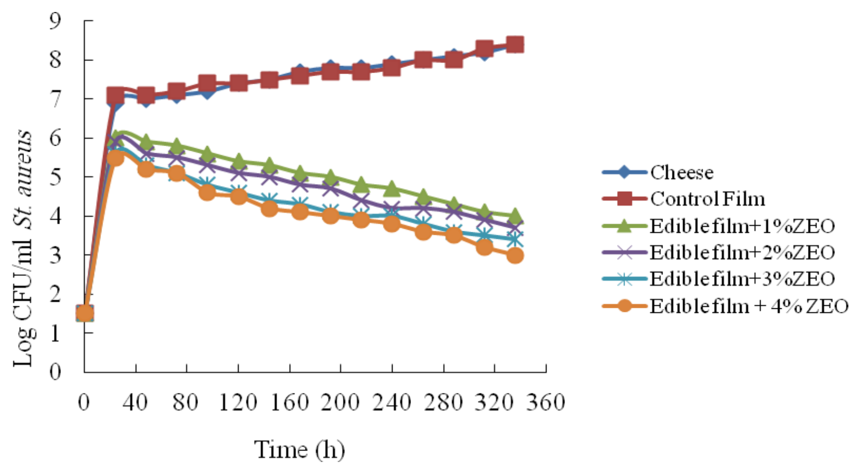

Figure 3. Inhibition of *Staphylococcus aureus* in cheese by zein edible film incorporated different concentrations of *Zataria multiflora* Boiss. essential oil.

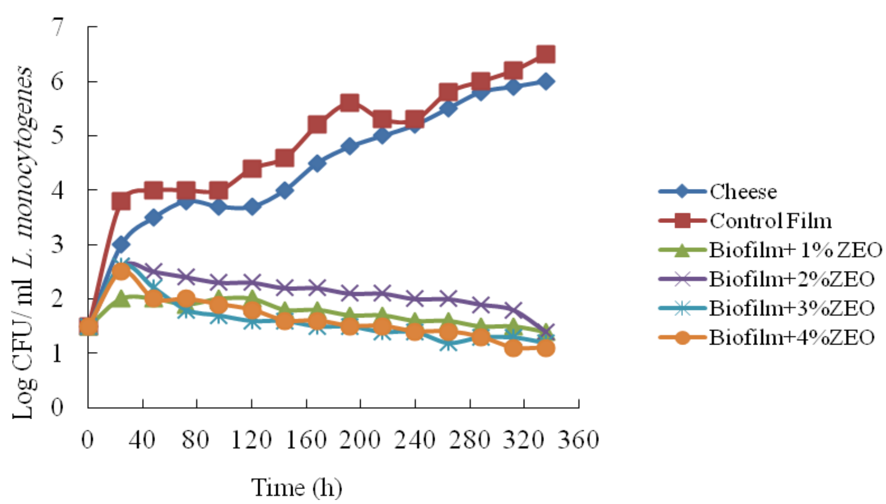

Figure 4. Inhibition of *Listeria monocytogenes* in cheese by zein edible film incorporated different concentrations of *Zataria multiflora* Boiss. essential oil.

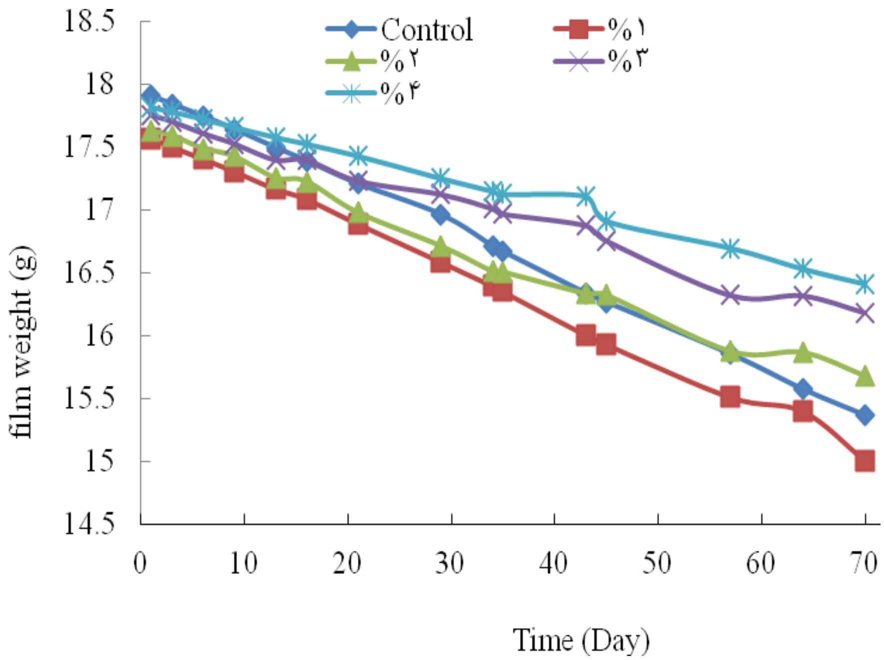

Figure 5. Water vapor transmission rate of zein films formulated with *Zataria multiflora* boiss. essential oil.

Water vapor transmission

Water vapor transmission rate (WVTR) is the steady state rate at which water vapor permeates through a film at specified conditions of temperature and relative humidity (Hu et al., 2001). The WVTR value was expressed in mg per day, of each container taken by the Equation (1):

$$(1 / N)[(WF - WI) - (CF - CI)]$$

in which N is the number of days expired in the test period (beginning after the initial 24 h equilibration period); ($WF - WI$) is the difference, in mg, between the final and initial weights of each test container; and ($CF - CI$) is the difference, in mg, between the average final and average initial weights of the controls, the data being calculated to two significant figures. Where the permeations measured are less than 5 mg per day, and where the controls are observed to reach equilibrium within 7 days, the individual permeations may be determined more accurately by using the 7-day test container and control container weights as *WI and CI*, respectively, in the calculation (Shimizu and Demarquette, 2000). Figure 5 shows water vapor transmission rate of zein films formulated with *Z. multiflora* Boiss essential oil (ZEO). The results show a decrease in water vapor transmission of films was observed as increasing amount of antimicrobial agent in Zein films solution. The zein films containing 4.00% (w/v) ZEO demonstrated minimum value of water vapor transmission (P<0.05).

DISCUSSION

Mechanical properties

It may be presumed that the films containing antimicrobial agent can affect the polymeric structure of the film and reduce the mechanical properties (Piers et al., 2008). The result was comparable with those of Ayana and Turhan (2009) who confirmed the plain films are stronger and more stretchable than the films which added some antimicrobial agents. In another study Piers et al. (2008) showed lower tensile strength and elongation to break for the films that containing antimicrobial agent than control films.

Antimicrobial activity

Results show a sharp decrease of the bacteria regarding the concentration of ZEO which is due to the presence of important phenolic components such as carvacrol and thymol. A case study by Kim et al. (1995) showed the antimicrobial effects of carvacrol on the *Salmonella* Typhimurium in culture medium and fish cube. They found that carvacrol with 3% concentration has very strong antimicrobial activity on the *S.* Typhimurium. In another study, Karaman et al. (2001) found the antimicrobial activity of the *T. revolatus* extract against some Gram positive and Gram negative bacteria. They concluded that it was because of the high quantity of the carvacrol in *T. revolatus*. Comparable studies were reported that the protein based films with ZEO consist of some phenolics competent that reduce free radicals for rising antimicrobial activities to some products. It may be because of the effect of higher intermolecular interaction, between phenolic compounds and protein films (Siripatrawan and Harte, 2012). There is evidence confirming the antibacterial activity of the zein film incorporated with 40 mg/ml Laurostearic acid, 0.0375 mg/ml nicin and 5058 mg/ml EDTA against *L. monocytogenes* and *S. enteritidis* (Hoffman et al., 2001). According to this study the number of *L. monocytogenes* declined from day 2 in plates containing zein films including Laurostearic acid or nicin whereas, the control film showed an increase. In another study Güçbilmez et al. (2007) found the effectiveness of zein film incorporated lysozyme, albumin protein and EDTA against *E. coli* and *Bacillus subtilis*. Del Nobile et al. (2008) found a significant difference between zein control films and zein films integrated thymol.

Water vapor transmission

The WVTR data showed that films prepared from ZEO revealed lower transmission rate than zein films. It appears that the essential oil of *Z. multiflora* containing hydrophobic antimicrobial compounds, could improve the moisture barrier properties (Moradi et al. 2012). According to Moradi et al. (2012) the chitosan films organized from ZEO showed lower transmission rate than chitosan film. In another study (Pintado et al., 2010) showed an increase of concentration of glycerol and sorbitol from 1.5 to 3.0% resulted in an increase in WVT. Hydroxyl groups of the plasticizers change polymer–polymer interactions by developing polymer-plasticizer hydrogen bonds, by this means increasing intermolecular spacing and transmission of film material (McHugh et al., 1994). According to Del Nobile et al. (2008) low gas permeability is frequently a great advantage in maintaining the quality of food products. This is classified with general preventative characteristics of plastics.

Conclusion

This study tries to use polymer material and natural antimicrobial agents for cheese packaging. All investigated features of ZEO formulated zein films were controlled by ZEO concentrations. WVT and antioxidant activities were improved after adding ZEO into zein film, while all films incorporated with antimicrobial agents showed lower strength and elongation values than the control films. These results might have application in cheese, which is effected by microbial spoilage. So, zein antimicrobial edible film might help the packaging system of the Iranian ultrafiltered Feta cheese. It could be concluded that plant extracts, with their powerful antimicrobial and antioxidant activity, may be the ideal biopreservative agents for cheese. The application of ZEO formulated zein films in cheese packaging was sufficient to prevent *S. enteritidis, L. monocytogenes, E. coli and S. aureus* growth and to conserve the overall quality and sensory attributes in treated cheese during 70 days storage. The proposed biopreservative agents incorporated in zein edible film could be innovatively applied for the preservation of dairy products.

Conflict of interests

The authors did not declare any conflict of interest.

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