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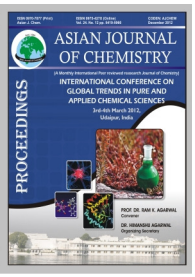

Investigation on Development of Zein Antimicrobial Edible Film and Essential Oil of *Zataria multiflora* Boiss. on *Sallmonella enteritidis, Listeria monocytogenes, Escherichia coli* and *Staphylococcus aureus*†

SARA GHASEMI[1], NASRIN HAJI SEYED JAVADI[1], MEHRAN MORADI[2], ABDOLRASOUL OROMIEHIE[3] and KIANOUSH KHOSRAVI-DARANI[1,*]

[1]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shaheed Beheshti Medical University. P.O. Box: 19395-4741 Tehran, Iran
[2]Department of Food Science and Technology, Ahar Faculty of Agriculture, University of Tabriz, 51664-16471, Tabriz, Iran
[3]Iran Polymer and Petrochemical Institute, Pajouhesh Blved, km-15, Tehran-Karaj Freeway

*Corresponding author: Fax: +98 21 22376473; Tel: +98 21 22376473; E-mail: kiankh@yahoo.com

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Zataria multiflora Boiss' essential oil is a natural product that would be able to effect on food-borne bacteria. In this research ethanol based antimicrobial zein edible films containing added 0.00, 0.50, 1.00, 1.50, 2.00 % (w/v) *Zataria multiflora* and glycerol as a plasticizer were created. The films were effective against *Salmonella enteritidis*, *Listeria monocytogenes*, *Escherichia coli* 0157: H7 and *Staphylococcus aureus.*

Key Words: Essential oil of *Zataria multiflora* Boiss., Zein edible film, *Salmonella enteritidis*, *Listeria monocytogenes*, *Escherichia coli* 0157: H7, *Staphylococcus aureus.*

INTRODUCTION

Active packaging is a type of packaging that can control or react to things arranged inside the packaging. In this case some types of active packaging will control the temperature inside the packaging while others control the moisture levels. It can also have systems that are antimicrobial, which extend the shelf life of their contents without the need of additives[1]. An antimicrobial active packaging is made by incorporating antimicrobial agents in food packages.

The film containing antimicrobial/antioxidant agents are a type of active packaging, which is designed mainly to control microbial and chemical spoilage of food. In this study, antimicrobial/antioxidant zein films are developed by incorporation of *Zataria multiflora* Boiss. essential oil (ZEO).

Zataria multiflora Boiss. is a plant that belongs to the *Lamiaceae* family and grows only in Iran, Pakistan and Afghanistan[2]. The antimicrobial activities of the plant are also well established against a wide variety of bacteria[3,4].

Ayana et al.[5] studied the antimicrobial effect of the methylcellulose (MC) films containing (% 0.5-3 w/v) olive leaf extract and glycerol (% 1.6 v/v). The results show the films were effective to decrease the amount of the *St. aureus* (ATCC 25923). The olive leaf extract in the films caused a reduce in the water vapour permeability and elongation (E), however an increase in tensile strength. Applying the file on Kasar cheese inoculated with *st. aureus*. The number of *St. aureus* declined logarithmically during 14th days.

In another study[6] found the antimicrobial effect of cellulose films incorporating of nicin, natamycin. *In vitro* situation the nicin films had the antimicrobial effect against *St. aureus* (ATCC 6538) and *L. monocytogenes* (ATCC 15313), while the natamycin films were effective against *Penicillium sp*. and *Geotrichum sp*. During nine days there was a decrease of *yeasts* and *moulds* on the cheese covered by natamycin at 12 ± 2 ºC. However the nicin films had antimicrobial effect against *psychrotrophic* during six days of the storage of the cheese. On the whole natamycin films were likely as active packaging for Mozzarella cheese.

The main aim of this study was to determine the antimicrobial activity of Zein edible film included essential oil of *Zataria multiflora* Boiss. in inhibiting *Listeria monocytogenes*, *Escherichia coli* O157: H7, *Salmonella enteritidis* and *Staphylococcus aureus*.

EXPERIMENTAL

Zataria multiflora Boiss. is purchased from Soha4 company, Iran. Fifty g dried leaves of *Zataria multiflora* is placed into a flask and the essential oil is extracted with a Clevenger-type apparatus using hydro-distillation method for 2 h.

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Salmonella enteritidis, Listeria monocytogenes, Escherichia coli 0157:H7 VT negative and *Staphylococcus aureus* were obtained from Microbiology Research Unit, Shahid Beheshti University, Tehran, Iran. These were maintained on nutrient agar (Oxoid CM0003) slopes at 5 ºC.

Preparation of inoculum: The above mentioned micro-organisms are transferred from the cultures to the slopes and incubated for 24 h in 37 ºC. After that the procedure for the three sub-cultures is carried out to make sure the organisms are active and vital. The organisms are sub cultured three times on consecutive days in nutrient broth (Oxoid CM0001) incubated at 37 ºC at precise 24 h intervals, followed by streaking on nutrient agar incubated at 37 ºC 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.

Preparation of films: The Zein Films are prepared according to casting method (Dry process). 5 g of Zein was dissolved in 45 mL of ethanol at 78 ºC. Glycerol was added (1.5 mL) as a plasticizer. Different concentration of *Zataria multiflora* Boiss. are added. After degassing, the films were cast on glass plates. Then dry overnight at laboratory temperature of course one of the films is without *Zataria multiflora*. It is as a control film[5].

For *in vitro* evaluation of the antimicrobial characteristics of the films the bacterial strains were transferred onto the surface of nutrient agar plates using sterile swabs and then discs of zein films containing different percent of the essential oil of the *Zataria multiflira* Boiss. put onto the surface of the plates. After that incubate over night at 37 ºC. Finally after 24 h the inhibitory zones is measured with caliper.

RESULTS AND DISCUSSION

There was a decline in the test organisms that was generally increased in the presence of the *Zataria multiflora* Boiss' essential oil. *Salmonella enteritidis, Listeria monocytogenes, Escherichia coli* 0157: H7 and *Staphylococcus aureus* showed significant reductions in bacterial survival in the higher extract concentration (Figs. 1 and 2).

Recently food industries are tending to use antimicrobial edible films to improve food safety and shelf life of product and sometimes it can be useful to improve the appearance and the taste of the product[7].

Many years ago food packaging just concerned and worried itself to be good-looking and efficient. Obviously nowadays packaging providers have to take the environmental impact of their products as well. In other words many types of packaging materials use more eco-friendly components to decrease the waste of the environment. Plastic packaging and coatings are always as the main environmental cause in the landfills. Packaging providers have focused on the production and sources of recyclable, eco friendly packaging. Plastics are made of long polymer molecules that are too large and too tightly bonded together to be broken down during composting. The bonds are permanent and hard to break. To get rid of this problem, researchers try to find ways of designing plastics that perform the necessary functions of food packaging but can be made to biodegrade in landfills after their use is finished[8].

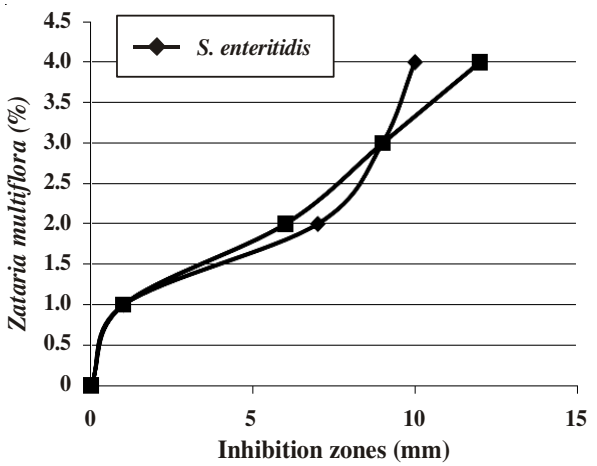

Fig. 1. Effect of 0, 1, 2, 3 and 4 % concentration of essential oil of *Zataria multiflora* in Zein edible films on the survival/growth of *S. enteritidis* and *E. Coli*

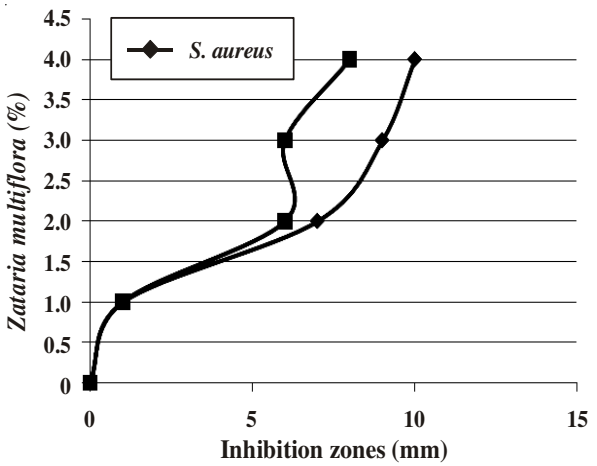

Fig. 2. Effect of 0, 1, 2, 3 and 4 % concentration of essential oil of *Zataria multiflora* in Zein edible films on the survival/growth of *S. aureus* and *L. monocytogenes*

In conclusion this study tries to use polymer material and natural antimicrobial agents. Besides nowadays researchers and industries are more interested in studying and using antimicrobial packaging, because it is able to control the quality and safety of the product.

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