



Scientific Research

## Production of edible film based on gelatin containing thyme essential oil: investigation of its physicochemical, mechanical, antioxidant and microbial properties

Rahimi, Z. 1, Hosseini, M. <sup>2\*</sup>, Saifi, T. <sup>2</sup>

1. Bachelor student of food hygiene

2. Assistant Professor, Department of Food Technology, Faculty of Agriculture, Ilam University, Ilam, Iran

3. Master of Veterinary Science, Ilam University, Ilam, Iran

### ABSTRACT

Considering the tendency to use biodegradable packaging films and increase their antimicrobial ability in recent years, this study aims to investigate different concentrations of thyme essential oil to improve edible film with physicochemical, mechanical, antioxidant and Antimicrobial was done. The effect of different concentrations of thyme essential oil for the production of edible films on the physicochemical, mechanical, antioxidant and antimicrobial properties was investigated with a completely randomized design in three replications and Duncan's multi-range test using Minitab18 software at a probability level of 0.05 were investigated. The highest tensile strength and elongation at break point was 2.5% in the treatment. In the treatment of films, the lowest solubility was observed at a concentration of 3.75% and permeability at a concentration of 2.5% of essential oil. In all treatments, the turbidity was significant, and in the concentration of 3.75% essential oil, the turbidity was the highest, and in the same concentration, the highest thickness was 0.13 mm, which was not significant in all treatments ( $p < 0.05$ ). In all edible film treatments, the antioxidant property using DPPH radicals was significant ( $p < 0.05$ ). The results of evaluating the antimicrobial activity of the film use of diffusion disks method showed that the largest diameter of the inhibition halo in the concentration of 3.75% was related to Staphylococcus aureus with an average halo diameter of 15.33 mm. Average halo diameter was reported for Pseudomonas aeruginosa and Escherichia coli, 8.6 mm and 9.52 mm, respectively. The general results showed that the addition of thyme essential oil at a concentration of 2.5% produced films that, in addition to inhibiting the growth and proliferation of bacteria, have sufficient strength and are also capable of being used in perishable food.

### ARTICLE INFO

#### Article History:

Received 2023/ 03/ 16

Accepted 2023/ 04/ 18

#### Keywords:

Thyme essential oil,  
Gelatin,  
Mechanical properties,  
Antioxidant.

DOI: 10.22034/FSCT.19.135.129

DOR: 20.1001.1.20088787.1402.20.135.11.7

\*Corresponding Author E-Mail:  
m.hosseini@ilam.ac.ir

## 1. Introduction

The use of plastic packaging to cover food due to its non-degradability and the occurrence of problems such as the migration of toxic chemicals such as bisphenol A, alkylphenol, octylphenol, phthalates, lead, etc. Therefore, among the coatings that have been widely considered in the food industry, we can mention edible composite films [2] and they are considered as one of the basic ways to control microbial and physicochemical changes in food [3]. Films containing gelatin have good mechanical resistance, and this material can be used in the production of edible film due to its gel-forming properties (the presence of proline and hydroxyproline amino acids) [4]. Gelatin can form an edible film to protect against drying, light and oxygen [5]. However, gelatin films have poor mechanical properties and water resistance [6]. One of the ways to improve the physicochemical properties of gelatin films is additives such as essential oils to strengthen its structure [7-11].

In order to reduce the use of antibiotics, chemical additives and preservatives, natural antimicrobial substances can be used in the composition of these packages [12]. However, the gelatin film has weak antimicrobial and antioxidant properties, and therefore it is not suitable for packaging perishable food and meat products [8]. In recent years, in research related to the production and evaluation of edible films, the use of essential oils (such as clove, cinnamon, ginger and basil essential oils) for microbial protection, postponing food spoilage (chemical and microbial) and reducing food waste has a significant place. have achieved [13-16].

In general, essential oils are volatile natural compounds that are secondary metabolites of plants that are listed because they are safe. GRAS Are. This plant is rich in mint tannins, polymethoxyflavones, triterpenes and polysaccharides. The most important compound in thyme essential oil is thymol, which has antimicrobial, antiviral, antifungal, anti-aflatoxin and antioxidant properties [17].

Bonilla et al. (2018) investigated the properties of eugenol and ginger essential oils in gelatin/chitosan films. Their results showed that a significant increase in the elasticity of all films was observed after the addition of active

compounds, while the water vapor permeability was largely unaffected [18].

Shahbazi et al. (2018) Antibacterial effect of gelatin film containing essential oil of Kakuti plant and grape seed extract against *Staphylococcus aureus* They investigated in ground beef. Their results showed that the number of bacteria *Staphylococcus aureus* In all the minced meat samples packed with gelatin films containing the essential oil of mountain kakuti and grape seed extract, it is significantly lower than the control group [19].

Ijaz et al. (2018) investigated the physicochemical properties of type B gelatin composite film containing clove essential oil and zinc oxide nanoparticles and its application in shrimp preservation. The results showed that the addition of nano particles caused the formation of a film with low flexibility and mechanical resistance [20].

Han et al. (2018) investigated the physical properties of sodium alginate and carboxymethylcellulose composite film containing cinnamon essential oil. Their research showed that cinnamon essential oil reduces thickness, permeability to water vapor and permeability to oxygen [21]. Wu et al. (2017) investigated the physicochemical properties and oil release of films containing cinnamon essential oil. The results showed that the tensile strength, elongation to breaking point, and moisture content of the gelatin-based film decreased with the increase in the concentration of essential oil, but its permeability to water vapor increased [22]. Langroudi et al. (2018) investigated the antimicrobial effect of sumac extract with edible chitosan coating containing Shirazi thyme essential oil on the preservation of beef packed with modified and conventional atmosphere. Their results showed that there was a significant decrease in the total count of bacteria, lactic acid bacteria, mold and yeast in all treatments compared to the control treatment during storage [23].

Considering that no research has been done on the antimicrobial effect and characteristics of the gelatin film containing thyme essential oil, the purpose of this research is to investigate the effect of thyme essential oil on its physicochemical, mechanical, and antioxidant properties due to its fragrance, antimicrobial properties, as well as

medicinal properties present in the plant. And microbial edible film is based on gelatin.

## 2- Materials and methods

### 2-1- Materials

Gelatin (Merck KGaA), chemicals and microbial culture media used in this research are manufactured by Merck. Thyme essential oil was prepared from local plants and from the Faculty of Agriculture of Ilam University. Microbial strains were obtained from the Faculty of Paraveterinary Medicine, Ilam University.

### 2-2- Methods

#### 2-2-1- essential oil extraction

Extraction of essential oil from thyme was done by distillation with water using Clonger machine for 3 hours at 90 degrees Celsius. Then the essential oil was collected and stored in a dark glass at a temperature of 4 degrees Celsius [24].

#### 2-2-2- Film production

Gelatin and thyme essence solutions were prepared separately. First, a 3% gelatin solution was prepared with gentle stirring (400 rpm) using a magnetic stirrer at a temperature of 50 degrees Celsius. Then 35% of glycerol plasticizer (based on the weight of the dry material used) was added and mixed for 15 minutes. In the next step, concentrations of (0, 1.25, 2.5, 3.75) percent of thyme essential oil were added to the gelatin solution and mixed together for 15 minutes. And after adding 0.2% Tween 80 as an emulsifier to the samples with a homogenizer (Universal, Germany), gelatin and glycerol solution were added and mixed for 20 minutes. Finally, the resulting solutions were poured into plates with a diameter of 15 cm and after complete drying (24-48 hours) at ambient temperature, the films were separated from the plate and placed inside aluminum foils in a desiccator containing saturated magnesium nitrate at a temperature of 25 degrees Celsius and relative humidity. 50% was placed for 72 hours to perform experiments [25].

#### 2-2-3- Measuring the thickness of films

The thickness of the produced films by micrometers (Mitutoyo Japan) was measured with an accuracy of 0.001 mm and at 10 different

points of each sample, and finally the average thickness was calculated and used to determine the tensile strength, permeability to water vapor and turbidity.]25[.

#### 2-2-4- film solubility

To determine the solubility, first, pieces of the film (cm<sup>2</sup>×cm<sup>2</sup>) were cut and placed in 50 ml of distilled water and stirred with a magnetic stirrer at a temperature of 25 degrees Celsius for 6 hours. The degree Celsius had reached constant weight, it was straightened and weighed (A). The filter paper with the sample was placed at 120 degrees Celsius and weighed again after reaching a constant weight (B). Finally, the solubility of films was calculated from the following equation]25[:

$$(A-B / A) \times 100 = \text{Solubility}$$

#### 2-2-5- Permeability to water vapor (permeability)

Permeability to water vapor was measured by gravimetric method (ASTM-E96-95, 1995). In a 14 ml container, 10 ml of distilled water was poured and covered by gelatin films with an area of 1.5 cm<sup>2</sup>. The capped bottle was first weighed and placed in a chamber containing silica gel with a certain relative humidity and temperature. The container was weighed every 12 hours for 3 days. Then the graph of changes in the weight of the dish was drawn against time. Its slope was calculated and the permeability to water vapor was calculated with the following formula]26[.

$$WVP^1(\text{gm}^{-1}\text{well}^{-1}\text{s}^{-1}) = \frac{W \times X}{A \times t \times \Delta p} \times \frac{W \times X}{A \times t \times \Delta p}$$

W the difference in the weight of the bottle, X the thickness of the gelatin film, A the area of the gelatin film (m<sup>2</sup>), t time in seconds and  $\Delta p$  The atmospheric vapor pressure difference contains thyme essential oil and pure water.

#### 2-2-6- Mechanical characteristics

The mechanical properties of the films include tensile strength (MPa), elongation to breaking point (%) and Young's modulus (MPa). These characteristics were calculated based on the ASTM-D882 standard and using a texture measuring device (TA-Plus, England) (ASTM2001). First, the movies are in cm<sup>2</sup> 1x10 were cut and the thickness was calculated at 10 points. The films were treated for 3 days in a

<sup>1</sup>.water vapor permeability

desiccator containing magnesium nitrate solution with a humidity of 55% and a temperature of 25°C, and then they were placed between two jaws of the histogram machine with an initial distance of 50 mm and a speed of movement of the jaw of 50 mm/min, and the mechanical characteristics included strength Tensile strength, the percentage increase in length to the breaking point (the change in the length of the sample divided by the initial length multiplied by 100) and also the Young's modulus were calculated from the force curve in terms of deformation.]25[.

#### 2-2-7- Antioxidant properties

The antioxidant activity of gelatin films was measured by radical color change from purple to yellow hydrazines. For this purpose, 30 mg of each film was dissolved in equal proportions of distilled water and methanol (ratio 3:3). After all the films were dissolved, it was centrifuged at 4000 rpm for 15 minutes (Universal, Germany). Then it was kept at 4°C for 12 hours (1 night rest) and centrifuged again for 15 minutes at 4000 rpm. Then, 1 ml of the upper part of the centrifuged solution of each film was mixed with 4 ml of 0.1 mM methanolic DPPH solution and kept in the dark for 30 minutes. Then the absorbance of each at 517 nm was measured by a spectrophotometer ( $A_s$ ). As a control, 1 ml of water and methanol solution (3 ml of distilled water + 3 ml of methanol) was incubated with 4 ml of 0.1 mM methanolic DPPH solution for 30 minutes in the dark in an incubator (Memmert, Germany) and absorbed. It was measured at 517 nm with a spectrophotometer (Shimadzu, Japan) ( $A_b$ ). Finally, the radical scavenging activity was calculated according to the following equation [25]:

$$\text{DPPH scavenging activity(\%)} = \frac{(A_b - A_s)}{A_b} \times 100$$

#### 2-2-8-Film opacity measurement

A spectrophotometer (Shimadzu, Japan) was used to determine the optical properties of the films. For this purpose, film samples were cut to 9x40 mm and placed inside the spectrophotometer cell. A blank cell sample was also considered as a control. The amount of light passing through different wavelengths and also its absorption at a wavelength of 500 nm was checked and calculated from the following formula to measure turbidity [25].

Film thickness / absorbance at 500 nm = film opacity

#### 2-2-9- Measurement of antimicrobial activity

From the agar disk diffusion method to determine the antimicrobial activity of films on bacteria *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* used. For this purpose,  $\mu\text{L}100$  (cfu/g<sup>6</sup>-10) From each of the bacterial suspensions, Mueller Hinton agar plates were inoculated. Then 6 mm discs were cut from each film and placed on its surface. Then they were kept in a greenhouse at a temperature of  $35 \pm 2$  degrees Celsius for 24 hours. Finally, the diameter of the growth inhibition zones (mm) was measured to determine the antimicrobial activity [25].

#### 2-2-10- Statistical analysis

All experiments were performed in three replicates ( $n=3$ ) with completely random sampling. One-way analysis of variance (ANOVA) and comparison of mean data based on Duncan's multiple range test using Minitab software<sup>18</sup> It was done at the probability level of 0.05.

## 3. Results and Discussion

### 3-1- Thyme essential oil analysis

Essential oils are complex compounds that can range from 20 to 60 compounds. Thyme essential oil compounds identified by GC-MS (Shimadzu, Japan) are listed in Table 1. The results showed that the dominant compound in thyme essential oil is thymol (39.44%). After that, P-cymene and Y-terpinene account for 23.6 and 12.51%, respectively.

Table1. Chemical composition of thyme essential oil

### 3-2- Mechanical test

The results of the mechanical tests of the combination of gelatin film with different concentrations of thyme essential oil, which indicate the maximum tensile stress and the hardness of the film and are related to its chains and internal links, are shown in Table 2, which are significant in all tests and the amount There were significant differences in tensile strength between different treatments ( $p < 0.05$ ). By increasing the percentage of essential oil, the mechanical indices increase compared to the control film (0%). The highest value of the test is related to the treatment of 2.5%, and with the increase in the percentage of essential oil, this trend decreases, which is probably due to the intermolecular bonding due to the influence of the plasticizer, and the tensile strength decreases [27]. The increase in length to breaking point (extensibility) in all samples has a significant difference ( $p < 0.05$ ) and has a greater value than the control treatment, and the highest value is 2.5% for the treatment, which is probably due to There is more water absorption and stronger hydrogen bonds in the gelatin protein structure, and the oil plasticizer increases the free volume in the sample, which is in the same

Composition	Value(%)
Thymol	39.44
P- Cymene	23.6
Y-Terpinene	12.51
Ledol	2.24
Aromadenrene	2.12
Caryophyllene	0.94
Farnesyl acetate	0.63
Linalyl acetate	0.55
-pinene $\alpha$	0.68
-thujone $\alpha$	0.52
Geanyl acetate	0.44
Bisabolene	0.27
3- eicosene	0.26
Farnesyl	0.52
Phutol	0.21
-pinene $\beta$	0.2
Thujanol	0.17
Camphor	0.17
Ethyl butyrate	0.63
Caryophyllene oxide	0.13
Nerolidol	0.077
Globulol	0.02
Hexadecanoic acid	0.019
7-tetradecene	0.032
Heptacosane	0.056
p-menth-2-in-1ol	0.10
5-a-pergn- 16-en-20 one	0.36
Octadecanoic methyl ester	0.14
Ethyl chrysanthemumate	0.005
2-pentadecanone6,10,14trimethyl	0.07

direction as the Young's model changes. The increase in the Young's model is probably because the essential oils are in the form of oil droplets. It can improve the elastic properties that occur as a result of increasing longitudinal changes compared to transverse changes in chains and compounds [21]. Several factors such as morphology and macromolecular structure, size and dispersion of particles as well as the degree of interaction between particles are effective in mechanical properties. In a study, Hosseini et al.(2013) investigated the characteristics of chitosan-gelatin polymers in different proportions. The

results showed that adding chitosan increased the tensile strength of the film [27].

Tongwanchan et al. (2016) in investigating the properties of fish skin gelatin film containing palm oil and basil essential oil with different surfactants found that the addition of essential oil decreased the tensile strength [16]. Wu et al. (2017) also obtained similar results during the investigation of fish gelatin films containing cinnamon essential oil. Due to the presence of different compounds in essential oils of different origins, these compounds are effective in interacting with protein in different ways [22].

**Table 2** Mechanical edible film properties

	Treatment	Tensile strength(Mpa)	Elongation at break(%)	Young modulus(Mpa)	Values with letters are different other based test at
different significantly from each on Duncan's (p<0.05)	0.00%	0.51 <sup>d</sup> ±3.59	0.02 <sup>d</sup> ±10.44	1.25 <sup>d</sup> ±26.5	R groups
	1.25%	0.2 <sup>b</sup> ±7.2	0.45 <sup>b</sup> ±15.14	2.01 <sup>b</sup> ±43.65	
	2.5%	0.14 <sup>a</sup> ±9.17	0.05 <sup>a</sup> ±19.37	1.55 <sup>a</sup> ±63.7	
	3.75%	0.32 <sup>c</sup> ±5.71	0.22 <sup>c</sup> ±13.33	1.42 <sup>c</sup> ±33.57	

### Physical properties of films prepared with different treatments (solubility, permeability to water vapor, turbidity and thickness)

The results of solubility, water vapor permeability, turbidity and thickness are shown in Table 3. No significant difference was observed in the properties of solubility value for control and 1.25% treatments (p<0.05), but the response of other concentrations was significant (p<0.05). The results showed that in the presence of different concentrations of thyme essential oil, the lowest solubility was related to treatments containing 3.75%. In general, the highest and lowest amount of solubility was related to control treatment and 3.75% treatment, respectively, so that it decreased significantly from 93.88% to 63.76% (p<0.05).

By increasing the concentration of essential oil, due to its oily nature, it is placed inside the network of biopolymers, so essential oil causes more mobility of the polymer network and as a barrier weakens the polymer network. The difference in solubility is dependent on the concentration and nature of the functional groups of the compounds in the composite films [14].

Adding essential oil to the composition of the film, depending on the type of chemical compounds as well as its hydrophobic nature, can cause the non-polar components of the essential oil to interact with the hydrophilic domain of gelatin and increase the hydrophobicity and decrease the solubility of the film [14]. Avlinajamriz et al. (2018) investigated the effect of lavender essential oil on the film of starch/forcellan/gelatin and found that the solubility of the films in water decreased with the increase in the amount of essential oil [12]. Mehrjahamd et al. (2018) also obtained similar results in the study of the effect of bergamot and lime essential oil on the film prepared from gelatin [28]. Adding cross-linking compounds to the gelatin film depending on the polarity of the

and also depending on the type of cross-linking compound can create hydrogen bonds, polar-polar, covalent or ionic bonds (salt bridge) [28-30].

The results of water vapor permeability showed that by adding thyme essential oil, the permeability decreased significantly in all treatments compared to the control film (p<0.05). The lowest rate of permeability was 2.5% of the film and the highest rate of permeability was the control film.

The penetration and passage of steam through the hydrophilic part of the film expresses the permeability to water vapor. The nature of biopolymers and added compounds, the structure of the biopolymer in the film matrix, the thickness and also the ratio of hydrophilic to hydrophobic in edible films have an effect on the degree of permeability to water vapor [6]. . The decrease in WVP can indicate an increase in the interaction and connections of thyme essential oil with gelatin polymer and creating a denser structure. Creating cross connections in the polymer matrix can reduce the free volume of the film matrix; It can also affect the orientation of the protein in the film structure and cause an increase in hydrophobic amino acids on the film surface and as a result change in the permeability of the film [18]. The excess amount of thyme essential oil, which is higher than the reactivity capacity of the gelatin polymer, can cause a heterogeneous structure and irregularity in the structure of the gelatin film. Also, by having a plasticizing role, thyme essential oil can increase molecular mobility and increase empty space in the film matrix [18]. Mehrjahamd et al. (2018) observed that mixing a higher level of bergamot essential oil increased the permeability, but a higher concentration of lime essential oil led to a decrease in the permeability of gelatin films [30], which they stated was the difference in the nature of the essential oils under investigation.

Turbidity is a measure to measure the transparency of films. The higher the turbidity,

the lower the transparency and can prevent oxidative spoilage in packaged foods. The comparison of the turbidity of the produced films showed that there is a significant difference between the turbidity of different treatments ( $p < 0.05$ ) and the high amount of turbidity is in films with a large amount of essential oil, which is probably related to the impurity level of gelatin and essential oil, which causes an increase in substances. The solid becomes soluble in water [22].

Similar to this study, the increase in the ratio of locust beans - gum made edible films cloudy. Contrary to the results of this research, increasing the level of glycerol in the film prepared from chickpea protein and Shirazi Gadomeh gum led

to a decrease in turbidity [14].

There is no significant difference in the thickness of the films between the control treatment and the 1.25% treatment, and it also follows this trend at higher concentrations of essential oil ( $p > 0.05$ ). The thickness of the film in the control treatment was 0.11 mm, and with the addition of essential oil, the thickness of the film increased significantly, so that the highest thickness of the films with the treatment of 3.75% of the essential oil treatment. The increase in the thickness of the films in the presence of essential oil is probably due to the reaction between the compounds in them. Of course, the placement of extract molecules in the film is also effective in increasing the thickness [14].

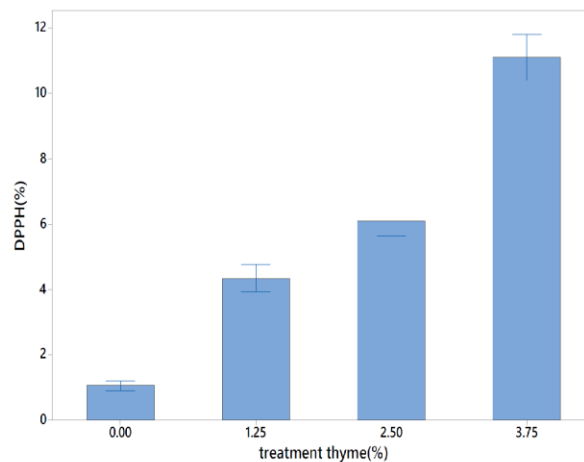
**Table 3** Physical properties of films

Treatment	Solubility(%)	Permeability( $\text{gm}^{-1}\text{s}^{-1}\text{well}^{-1}$ )	Turbidity	Thickness(mm)
0.00%	1.4 <sup>a</sup> ±93.88	0.02 <sup>a</sup> ±1.6	0.16 <sup>d</sup> ±0.12	0.001 <sup>b</sup> ±0.11
1.25%	1.99 <sup>a</sup> ±91.51	0.02 <sup>b</sup> ±1.23	0.01 <sup>c</sup> ±0.92	0.005 <sup>b</sup> ±0.11
2.5%	2.81 <sup>b</sup> ±74.4	0.1 <sup>c</sup> ±8.3	0.17 <sup>b</sup> ±1.25	0.007 <sup>a</sup> ±0.12
3.75%	3.82 <sup>c</sup> ±63.76	0.39 <sup>c</sup> ±9.4	0.04 <sup>a</sup> ±1.78	0.001 <sup>a</sup> ±0.13

Values with different letters are significantly different from each other based on Duncan's test at ( $p < 0.05$ )

### 3-4-Antioxidant properties

Figure 1 shows that with the increase of essential oil concentration, the antioxidant activity of the treatments increased significantly compared to the control film ( $p < 0.05$ ). The results showed that all the studied treatments had the ability to inhibit free radicals. In the Shahid treatment film, an average value of 1.05% of antioxidant activity was observed, which could be due to the presence of certain amino acids such as glycine and proline in gelatin, as well as the presence of cyclic amino acids, tryptophan, phenylalanine, and tyrosine [31]. Ramzi et al. (2011) The weak antioxidant activity of the essential oil of *Bacillus* species to reduce the DPPH radical was attributed to its low content of phenolic compounds [32]. Akhtar et al. (2019) reported that the nature of the film and the difference in the antioxidant power of the added compounds have an effect on the antioxidant activity of the films [33].



**Fig 1** Antioxidant properties of control film and films with different concentrations of thyme essential oil

### 5-3- Antimicrobial activity

The effect of different films of thyme essential oil on reducing or preventing the growth of microorganisms *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was investigated by agar diffusion method. The amount of microorganism growth inhibition (diameter of non-growth halo) is shown in Table 4. The results showed that in the control film, little antimicrobial activity was observed against

all three studied species, which can be ignored, but the addition of different concentrations of thyme essential oil to the gelatin film structure inhibited the growth of all three studied bacterial species. Increasing the concentration of thyme essential oil had a significant effect on increasing the diameter of the growth halo of the investigated microorganisms ( $p < 0.05$ ). The highest rate of growth inhibition in all three bacterial species *Pseudomonas aeruginosa* and *Escherichia coli* in the treatment of 1.25% and bacteria *Staphylococcus aureus* in the treatment, it was 3.75%. To *Staphylococcus aureus* with the increase of essential oil concentration, the rate of growth inhibition increased so that the maximum diameter of the halo of no growth was 15.33 mm corresponding to the 3.75% treatment.

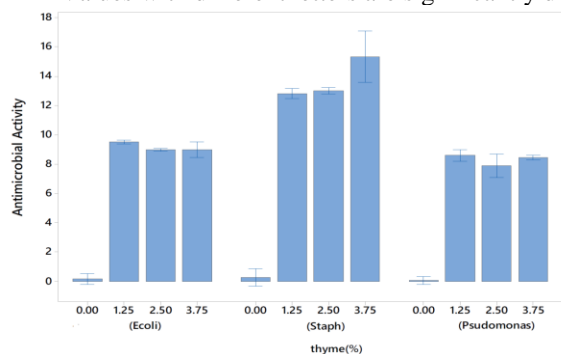
The diagram of the inhibition rate of the growth of microorganisms (diameter of the halo of non-growth) is shown in Figure 2. The results showed that in the control film, the antimicrobial activity against all three studied species is similar and negligible. Antimicrobial activity of treatments containing thyme essential oil can be the result of synergistic effect of monoterpene hydrocarbons and its derivatives. Studies have shown that minor ingredients (with low concentration) in the composition of films can play an important role

in the antimicrobial activity of films [30]. Rehana Akhtar et al. (2019) in the study of biocomposite films containing rosemary and mint essential oils, found the inhibition of the growth of both Gram-positive and Gram-negative bacteria groups to be the result of the presence of polyphenolic compounds [33]. Essential oils through various mechanisms including: disruption of phospholipid cell membrane and cytoplasm leakage, reaction with cell membrane respiratory enzymes as well as inhibition of enzyme synthesis in mitochondria, effect on genetic material and nuclear compounds by electrophilic compounds, reduction of energy in microbial cells due to release of the protonation of hydroxyl groups or the formation of hydroperoxidase fatty acids (resulting from the oxygenation of unsaturated fatty acids) can affect microorganisms. In general, the effectiveness of the edible film against microbial growth depends on the nature of the essential oil and the type of microorganism. Therefore, it can be concluded that due to inhibiting the growth of both Gram-positive and Gram-negative bacteria groups, gelatin composite films can be effective for food preservation and increase the shelf life and maintain the quality and safety of the product [25].

**Table 4** The antimicrobial activity (diameter of the growth inhibition zone (mm)) of the combined films of thyme essential oil on the tested model microorganisms

Treatment	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
0.00%	0.01 <sup>c</sup> ±0.07	0.24 <sup>b</sup> ±0.27	0.14 <sup>b</sup> ±0.17
1.25%	0.15 <sup>b</sup> ±8.6	0.13 <sup>c</sup> ±12.82	0.045 <sup>c</sup> ±9.52
2.5%	0.32 <sup>a</sup> ±7.9	0.09 <sup>c</sup> ±13	0.35 <sup>a</sup> ±8.99
3.75%	0.06 <sup>b</sup> ±8.44	0.7 <sup>a</sup> ±15.33	0.21 <sup>a</sup> ±8.89

Values with different letters are significantly different from each other based on Duncan's test at ( $p < 0.05$ )



**Fig 2** Antimicrobial activity of gelatin films with different concentrations of thyme essential oil

## 5- Resources

[1] Al-Hassan, A., Norziah, M. 2012. Starch-

- gelatin edible films: water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, 26(1):108-17.
- [2] Piermaria, JA., Pinotti, A., Garcia, MA., Abraham, AG. 2009. Films based on kefir, an exopolysaccharide obtained from kefir grain: development and characterization. *Food Hydrocolloids*, 23(3):684-90.
- [3] Sorrentino, A., Gorrasi, G., Vittoria, V. 2007. Potential perspectives of bio-nanocomposites for food packaging applications. *Trends in Food Science & Technology*, 18(2):84-95.
- [4] Gennadios, A. 2002. Protein-based films and coatings: CRC Press.
- [5] Taghizadeh, M., Mohammadifar, MA.,



- Sadeghi, E., Rouhi, M., Mohammadi, R., Askari, F. 2018. Photosensitizer-induced cross-linking: A novel approach for improvement of physicochemical and structural properties of gelatin edible films. *Food research international*, 112:90-7.
- [6] Jridi, M., Hajji, S., Ayed, HB., Lassoued, I., Mbarek, A., Kammoun, M. 2014. Physical, structural, antioxidant and antimicrobial properties of gelatin-chitosan composite edible films. *International journal of biological macromolecule*, 67:373-9.
- [7] Sahraee, S., Milani, JM., Ghanbarzadeh, B., Hamishehkar, H. 2017. Effect of corn oil on physical, thermal, and antifungal properties of gelatin-based nanocomposite films containing nano chitin. *LWT-Food Science and Technology*, 76:33-9.
- [8] Cheng, S., Wang, W., Li, YGao.G., Zhang, K., Zhou, J. 2019. Cross-linking and film-forming properties of transglutaminase-modified collagen fibers tailored by denaturation temperature. *Food chemistry*, 271:527-35.
- [9] Alves, PM., Carvalho, RA., Moraes, IC., Luciano, CG., Bittante, AMQ., Sobral, PJ. 2011. Development of films based on blends of gelatin and poly (vinyl alcohol) cross linked with glutaraldehyde. *Food hydrocolloids*, 25(7):1751-7.
- [10] Chiou, B-S., Avena-Bustillos, RJ., Bechtel, PJ., Jafri, H., Narayan, R., Imam, SH. 2008. Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. *European Polymer Journal*, 44(11):3748-53.
- [11] Tomadoni, B., Ponce, A., Pereda, M., Ansorena, M. 2019. Vanillin as a natural cross-linking agent in chitosan-based films: Optimizing formulation by response surface methodology. *Polymer Testing*, 10(5):93-95.
- [12] Jamróz, E., Juszczak, L., Kucharek, M. 2018. Investigation of the physical properties, antioxidant and antimicrobial activity of ternary potato starch-furcellaran-gelatin films incorporated with lavender essential oil. *International journal of biological macromolecules*. 114:1094-101.
- [13] Echeverría, I., López-Caballero, ME., Gómez-Guillén, MC., Mauri, AN., Montero, MP. 2018. Active nanocomposite films based on soy proteins-montmorillonite-clove essential oil for the preservation of refrigerated bluefin tuna (*Thunnus thynnus*) fillets. *International journal of food microbiology*, 266:142-9.
- [14] Ahmed, J., Mulla, M., Arfat, YA., Bher, A., Jacob, H., Auras, R. 2018. Compression molded LLDPE films loaded with bimetallic (Ag-Cu) nanoparticles and cinnamon essential oil for chicken meat packaging applications. *LWT*, 93:329-38.
- [15] Bonilla, J., Poloni, T., Lourenço, RV., Sobral, PJ. 2018. Antioxidant potential of eugenol and ginger essential oils with gelatin/chitosan films. *Food bioscience*. 23:107-14.
- [16] Tongnuanchan, P., Benjakul, S., Prodpran, T., Pisuchpen, S., Osako, K. 2016. Mechanical, thermal and heat sealing properties of fish skin gelatin film containing palm oil and basil essential oil with different surfactants. *Food Hydrocolloids*. 56:93-107.
- [17] Prakash, B., Mishra, PK., Kedia, A., Dubey, N. 2014. Antifungal, antiaflatoxin and antioxidant potential of chemically characterized *Boswellia carterii* Birdw essential oil and its in vivo practical applicability in preservation of *Piper nigrum* L. fruits. *LWT-Food Science and Technology*, 56(2):240-7.
- [18] Bonilla, J., Poloni, T., Lourenço, RV., Sobral, PJ. 2018. Antioxidant potential of eugenol and ginger essential oils with gelatin/chitosan films. *Food bioscience*. 23:107-14.
- [19] Shahbasi, Y., Mosavi, MH. 2016. Study on the effect of *Ziziphora clinopodioides* essential oil and methanolic pomegranate peel extract on physical, mechanical and antibacterial properties of edible chitosan film. *Food technology research journal*. 27(2): 121-134.
- [20] Ejaz, M., Arfat, YA., Mulla, M., Ahmed, J. 2018. Zinc oxide nanorods/clove essential oil incorporated Type B gelatin composite films and its applicability for shrimp packaging. *Food Packaging and Shelf Life*, 15:113-21.
- [21] Han, JH 2003. Antimicrobial food packaging. *Novel food packaging techniques*, 8:50-70.
- [22] Wu, J., Sun, X., Guo, X Ge S., Zhang, Q. 2017. Physicochemical properties, antimicrobial activity and oil release of fish gelatin films incorporated with cinnamon

- essential oil, *Aquaculture and Fisheries*, 2(4):185-92.
- [23] Langroodi, AM., Hossein, Tajik. 2017. Antimicrobial effects of hydroalcohol sumac extract with chitosan containing zataria multiflora boiss essential oil on beef meat in normal and modified atmosphere packaging. *The Journal of Urmia University of Medical Sciences*, 28(3):192-205
- [24] European Commission Regulation No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives Text with EEA relevance.
- [25] Fallah, M., Rouhi, M., Sadeghi, E., Sarlak, Z., Moghadam, A & Mohammadi, R. 2021. Effects of Olibanum essential oil on physicochemical, structural, antioxidant and microbial characteristics of Gelatin edible films. *Iranian journal of nutrition science and food technology*, 15(4): 93-102.
- [26] Zafarani, M., Ojagh, SM., Alishahi, A., Kazemi, M. 2017. The effect of chitosan type on the properties of biodegradable films produced from gelatin obtained from the skin of elephant fish. *Quarterly journal of new food technologies*, 6(1): 149-159.
- [27] Hosseini, SF., Rezaei, M. 2016. Development of flexible bactericidal films based on poly (lactic acid) and essential oil and its effectiveness to reduce microbial growth of refrigerated rainbow trout. *LWT-Food Science and Technology*, 72:251-60.
- [28] Ahmad, M., Benjakul, S., Prodpran, T., Agustini, TW. 2012. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. *Food Hydrocolloids*, 28(1):189-99.
- [29] Hanani, ZN., Roos, YH., Kerry, J. 2014. Use and application of gelatin as potential biodegradable packaging materials for food products. *International journal of biological macromolecules*, 71:94-102.
- [30] Martucci, JF., Gende, LB., Neira, L., Ruseckaite, R.A. 2015. Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films. *Industrial Crops and Products*, 71:205-13.
- [31] Alexandre, EMC., Lourenço, RV., Bittante, AMQB., Moraes, ICF. 2016. Gelatin-based films reinforced with montmorillonite and activated with nanoemulsion of ginger essential oil for food packaging applications. *Food Packaging and Shelf Life*, 10:87-96.
- [32] Mothana, RA., Hasson, SS., Schultze W., Mowitz, A., Lindequist, U. 2011. Phytochemical composition and in vitro antimicrobial and antioxidant activities of essential oils of three endemic Soqotraen *Boswellia* species. *Food chemistry*, 126(3):1149-54.
- [33] Akhter, R., Masoodi, F., Wani, TA., Rather, SA. 2019. Functional characterization of biopolymer based composite film: Incorporation of natural essential oils and antimicrobial agents. *International journal of biological macromolecules*, 137:1245-55.



تولید فیلم خوراکی بر پایه ژلاتین حاوی اسانس آویشن: ارزیابی خصوصیات فیزیکوشیمیایی، مکانیکی،

آنتی اکسیدانی و میکروبی

زینب رحیمی<sup>۱</sup>، محمدیار حسینی<sup>۲\*</sup>، طیب سیفی<sup>۳</sup>

۱- دانشجوی کارشناسی بهداشت مواد غذایی.

۲- استادیار گروه صنایع غذایی، دانشکده کشاورزی، دانشگاه ایلام، ایلام، ایران.

۳- کارشناسی ارشد باکتری شناسی دامپزشکی، دانشگاه ایلام، ایلام، ایران.

## چکیده

## اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۱/۱۲/۲۵

تاریخ پذیرش: ۱۴۰۲/۰۱/۲۹

کلمات کلیدی:

اسانس آویشن،

ژلاتین،

خواص مکانیکی،

آنتی اکسیدانی

با توجه به تمایل استفاده زیاد فیلم های بسته بندی زیست تخریب پذیر و افزایش قابلیت ضد میکروبی آنها در سال های اخیر، این مطالعه با هدف بررسی غلظت های مختلف اسانس آویشن جهت بهبود فیلم خوراکی با ویژگی های فیزیکوشیمیایی، مکانیکی، آنتی اکسیدانی و ضد میکروبی انجام گرفت. اثر غلظت های مختلف اسانس آویشن برای تولید فیلم های خوراکی روی ویژگی های فیزیکوشیمیایی، مکانیکی، آنتی اکسیدانی و ضد میکروبی با طرح کاملاً تصادفی در سه تکرار و آزمون چند دامنه ای دانکن با استفاده از نرم افزار Minitab18 در سطح احتمال ۰/۰۵ مورد بررسی قرار گرفتند. بالاترین میزان استحکام کششی و ازدیاد طول در نقطه شکست در تیمار ۲/۵٪ بود. در تیمار فیلمها کمترین میزان حلالیت در غلظت ۳/۷۵٪ و نفوذپذیری در غلظت ۲/۵٪ اسانس مشاهده شد. در تمامی تیمارها کدورت معنی دار بود و در غلظت ۳/۷۵٪ اسانس، کدورت بیشترین مقدار را به خود اختصاص داد و در همین غلظت نیز بیشترین ضخامت mm 0/13 بدست آمد که در همه تیمارها معنی دار نبود ( $p > 0/05$ ). در تمامی تیمارهای فیلم خوراکی خاصیت آنتی اکسیدانی با استفاده از رادیکالهای DPPH معنی دار بود ( $p < 0/05$ ). نتایج ارزیابی فعالیت ضد میکروبی فیلم با کمک روش دیسک های انتشاری نشان داد که بیشترین قطر هاله بازدارندگی در غلظت ۳/۷۵٪ مربوط به *Staphylococcus aureus* با متوسط قطر هاله mm 15/33 بود. متوسط قطر هاله برای *Pseudomonas aeruginosa* و *Escherichia coli* به ترتیب mm 8/6 و mm 9/52 گزارش شد. نتایج کلی نشان داد که افزودن اسانس آویشن در غلظت ۲/۵ درصد باعث تولید فیلم هایی گردید که علاوه بر مهار رشد و تکثیر باکتریها، دارای استحکام کافی بوده و همچنین قابلیت استفاده در مواد غذایی فسادپذیر را دارد.

DOI: 10.22034/FSCT.19.135.129

DOR: 20.1001.1.20088787.1402.20.135.11.7

\* مسئول مکاتبات:

m.hosseini@ilam.ac.ir