

# Physico-Mechanical and Antimicrobial Properties of Isolated Soy Protein Film Incorporated with Peppermint Essential Oil on Raw Hamburger

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## ABSTRACT

Meat products are highly perishable and require protection to maintain their quality. Bioedible films incorporated with essential oils have recently received attention due to their benefits as AntiMicrobial (AM) active packaging. The aim of this study was to evaluate the physico-mechanical properties and antimicrobial activity of Isolated Soy Protein (ISP) film containing Peppermint Essential Oil (PEO) on shelf life and sensory quality of raw hamburger. The ISP film incorporated with PEO at 1, 2, and 3% (v/v) were prepared by solvent casting method. The results showed that the incorporation of PEO caused a significant decrease and an increase ( $P < 0.05$ ) in tensile strength and elongation-at-break, respectively. Increment of thickness, Water Vapor Permeability (WVP), and decline in moisture content was recorded as the amount of oil increased ( $P < 0.05$ ). In addition, Lightness/darkness ( $L^*$ ), Whiteness Index (WI), and redness/greenness ( $a^*$ ), and yellowness/blueness ( $b^*$ ) increased, while the total color difference ( $\Delta E$ ) decreased by adding PEO. PEO-incorporated film (at 3% v/v) exhibited higher inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, and lower for *Salmonella enterica* using disc diffusion method. Microbial analysis and pH measurement of raw hamburger covered with ISP-PEO film showed no inhibitory effect against test bacteria when applied on raw hamburger, whereas the inhibited total bacterial growth exceeded the acceptable limit until the end of refrigerated storage. This film was able to prolong the shelf life of hamburger for up to 7 days. Therefore, this new antibacterial film has considerable potential to be used as meat packaging material.

**Keywords:** Antimicrobial activity, Bacterial growth, Bioedible film, Hamburger shelf life.

## INTRODUCTION

Meat and meat products provide all of the essential amino acids for human body, but they are susceptible to chemical deterioration and microbial spoilage (Ortega *et al.*, 2014). According to World Health Organization (WHO) estimates, nearly one in every ten people around the world is infected by foodborne pathogens each year, and one of the most common sources of fatal infections is meat (World Health Organization, 2016). In recent years, the consumption of ready-to-eat meat products

and fast foods has increasingly become popular due to changes in human lifestyle and eating habits. Hamburger is one of the most common fast foods in the world and is growing in popularity with consumers; opting for greater convenience. In addition, there is an increasing interest in the study of meat products including maintaining product quality and extending product shelf life throughout transport and storage period (Theivendran *et al.*, 2006; Kerry *et al.*, 2006).

Edible film is defined as a preformed thin layer or solid sheet of edible material that can be used on or between food components

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to protect perishable food products from deterioration and quality loss. They can provide an alternative for extending the shelf life of meat products by having biochemical and physicochemical stability and acting as barriers to moisture or humidity, oxygen, and carbon dioxide. In general, edible films are derived from protein, polysaccharide or lipid. Protein-based materials are generally preferred to polysaccharides because of their ability to form films with high mechanical and barrier properties and they provide higher nutritional value. In addition, they can be used for controlled release of antimicrobial compounds (Janjarasskul and Krochta, 2010; Pascall and LinS, 2013). Thus, many studies have been focused on producing films from different protein sources such as whey, soy, Wheat Gluten (WG), pea (Galiotta *et al.*, 1998; Vo Hong *et al.*, 2015; Sun *et al.*, 2013). Isolated Soy Protein (ISP) shows striking potential as a packaging material due to its innate advantages of biodegradability, biocompatibility, film-forming capacity, mechanical and barrier (water vapor) properties (Saremnezhad *et al.*, 2011; Zink *et al.*, 2016).

Edible films can also be impregnated with various kinds of antimicrobial agents in order to enhance their functionality. In this case, plant essential oils are gaining a wide interest in food industry for their broad spectrum of antimicrobial activity against food-borne pathogens and their approval by Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS). The enhanced antimicrobial activity of edible film blended with essential oil may result from the ability of the essential oils and their components to dissolve the lipids of the bacterial cell membrane while disorganizing the structures and causing more permeability due to the hydrophobic nature of the essential oil (Nazzaro *et al.*, 2013). Peppermint (*Mentha piperita*) Essential Oil (PEO) is one of the most effective essential oils that has been pointed out to possess antimicrobial activity for meat applications, which could be attributed to the presence of

active compounds such as, menthol and menthone (Mahboubi and Kazempour, 2014).

This research focused on evaluating the incorporation of different concentrations of PEO in ISP based edible film to improve the physico-mechanical properties and antimicrobial activity against inoculated *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, and the microbiota developed at different times of refrigerated storage of raw hamburger.

## MATERIALS AND METHODS

### Raw Materials and Bacterial Strains

Isolated soy protein with 90% protein content was supplied by Shandong Company (China) and aerial parts of *Mentha piperita* were collected from a field located in the Darab city (1,181 m above mean sea level, latitude 29° 68' N and altitude 53° 2' E.) in Fars Province, Iran. *Staphylococcus aureus* PTCC 1113, *Salmonella enterica* PTCC 1709 and *Escherichia coli* PTCC 1399 cultures were purchased from the culture collection of Iranian Organizations for Science and Technology (IROST).

### Preparation of Soy Protein Isolate-Based Film

ISP based edible film was prepared according to Choi *et al.* (2003) and Ghasemloua *et al.* (2013). PEO was incorporated into the film solution in different concentrations (1, 2, 3, 4%, and 5% v/v) by using tween 80 (0.1% v/v) for maintaining homogeneous distribution of oil in the solution. The film solution was casted onto sterile plastic Petri dishes (9 mm diameter) and allowed to dry in oven at 30°C for 72 hours. Films were peeled and stored in desiccators at 25°C and 53% Relative Humidity (RH) until evaluation. Homogeneous films with no phase separation, without insoluble particles, and

uniform color checked visually were selected for the analytical determinations.

### Preparation of *Mentha piperita* (Peppermint) Essential Oil

One hundred g dried leaves of plant were placed into a flask, and the essential oil extraction was performed with a Clevenger-type apparatus using hydro-distillation method for 4 hours. The essential oil was collected and dried with anhydrous sodium sulfate and stored in sealed vials at 4°C (Mahboubi and Kazempour, 2014).

### Gas Chromatography (GC-Mass) Analysis of Peppermint Essential Oil

The GC analysis was performed using a Shimadzu GC-9A coupled with CR4A integrator. A Thermon 600T fused silica capillary column (50 m×0.25 mm, 0.20-μm film thickness) and nitrogen as carrier gas were used. Oven temperature was programmed as follows; kept at 70°C for 10 minutes, raised to 180°C at a rate of 2°C min<sup>-1</sup> and then kept at 180°C for 30 minutes. Injector and detector temperatures were 250°C. Relative percentage levels of the separated compounds were calculated from FID (Flame Ionization Detector) chromatograms.

### Characterization of Antimicrobial Film

The films incorporated with various concentrations of PEO (0-3%) were characterized by physico-mechanical analysis; thickness, moisture content, water vapor barrier properties, tensile strength, and elongation at break.

### Film Thickness

Film thickness was measured using a digital micrometer (0-25 – Japan) at ten different locations, and the mean of measurements

was defined as a film thickness (Ghasemloua *et al.*, 2013). The thickness of an average film was used for further studies.

### Moisture Content

The moisture content of films was measured by oven drying the film at 110°C until constant weight. The weight loss of each sample was determined, and the moisture content was calculated as the percentage of water removed from the system (Shojaee *et al.*, 2013).

### Water Vapor Permeability (WVP)

Water Vapor Permeability (WVP) of ISP based edible films was measured as described by Pires *et al.* (2013). The WVP was calculated as follow:

$$WVP = (WVTR \times L) / \Delta P \quad (1)$$

Where, *WVTR* is the measured Water Vapor Transmission Rate (g m<sup>-2</sup> s<sup>-1</sup>) through a film, *L* is the mean film thickness (mm),  $\Delta P$  is the water vapor Pressure difference (kPa) between the two sides of the film.

### Surface Color Measurement

Color measurements of the films samples were carried out using a colorimeter (Pansharpen-48A, China). The Hunter color scale was used to measure Lightness/darkness (*L\**), chromaticity parameters *a\** (red–green) and *b\** (yellow–blue). Measurements were performed by placing the film sample over the standard white plate (*L\**= 55.38, *a\**= -3.44 and *b\**= -0.041). Total color differences ( $\Delta E$ ) and Whiteness Index (WI) were calculated with respect to standard plate parameters by using the following equations (Tongnuanchan *et al.*, 2014):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (2)$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (3)$$



Where,  $L^*$ ,  $a^*$  and  $b^*$  are the color parameter values of the standard and  $L$ ,  $a$ , and  $b$  are the color parameter values of the sample.

### Tensile Strength and Elongation at Break

The moderate film thickness was placed into a texture analyzer (Gotech, Taiwan) to measure tensile strength (Mpa) and percentage elongation at break (%) according to the ASTM (American Society for Testing and Materials) Standard Method D882-00 (2000) (Praseptianga *et al.*, 2016). The tensile strength and the percentage elongation at break were calculated using the following equations. All of the results were the means of at least three measurements.

Tensile strength (MPa)= (Maximum load)/(Original minimum cross section area) (4)

%Elongation= (Extension at moment of rupture)/(Initial gage length) (5)

### Antimicrobial Activity of the Edible Film Incorporated with Essential Oil

Antibacterial activity of the prepared films containing 1-3% of PEO was evaluated against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* using agar disc diffusion method according to Balouiri *et al.* (2016). Finally, film with the highest antimicrobial activity was selected for further study. All analyses were conducted in triplicate.

### Effect of Antimicrobial Edible Film on Natural Background Flora of Raw Hamburger

According to the findings of the antibacterial assay and considering the possible diffusion of essential oils from the film matrix into the hamburger texture, the film containing the

most effective PEO concentration (3%) was prepared in order to evaluate its ability to control residing microbial growth and, subsequently, to extend the shelf life of raw hamburgers. Therefore, the raw hamburgers were divided into three groups. The first group was assigned as control and was unwrapped. The second and the third groups were wrapped on the upper and the bottom surfaces with ISP based films with and without PEO, respectively. The treatments were placed individually into sterile Petri plates and refrigerated at 4°C for 12 days. Sampling was performed at days 0, 1, 3, 5, 7, and 12 for evaluation of microbial quality as well as pH value. It should be noted that the microbial quality of raw hamburgers of this study were in accordance with Iranian National Standard limits No.2304 (3<sup>rd</sup> revision).

### Antimicrobial Effect of Edible Film on Surface Inoculated Raw Hamburgers

The hamburger samples were inoculated artificially with a diluted overnight culture (0.1 mL; 10<sup>6</sup> CFU mL<sup>-1</sup>) of 3 bacterial strains, *E. coli*, *S. aureus*, *S. enterica*. The inoculated samples were then dried under the bio-hood for 30 minutes. Finally, the samples were surface wrapped with ISP based film with and without PEO, separately. The inoculated wrapped samples were stored at 4°C for 12 days, and sampling were done at days 0, 1, 3, 5, 7 and 12 for plating and enumeration of survivors. In addition, an inoculated unwrapped hamburger sample was included as control and stored along with test samples. The pH measurements were carried out at days 0, 1, 3, 5, 7 and 12 of refrigerated storage.

### Microbiological Analysis

At each sampling interval, 5 g of each sample were homogenized with 45 mL of 0.1% Buffered Peptone Water [BPW; Merck (Cat NO;1072280500), Darmstadt,

Germany] in a stomacher and diluted using decimal dilution for plating onto selective agar including plate count agar (total aerobic bacteria), Macconkey agar (*E. coli*), Baird Parker agar (*S. aureus*), Salmonella Shigella agar (*S. enterica*). All plates were incubated at  $37\pm 2.00^{\circ}\text{C}$  for 24 hours. Bacterial counts were expressed as log CFU  $\text{g}^{-1}$  (Iranian National Standard, No. 2304, No. 1- 10899, No. 9263, No. 1810).

### Organoleptic Analysis

Organoleptic analysis was carried out by a group of 6 semi-trained panelists using a 9-point hedonic scale ranging from “very strong like, score 9” to “very strong dislike, score 1”. Score of 1–9 was assigned for the overall acceptability of the precooked samples (control, wrapped with and without PEO), which was determined by assessing the appearance, color, odor, taste, texture and flavor. The organoleptic score of 5 was considered as the lower limit of acceptability (Meilgaard *et al.*, 1999).

### Statistical Analysis

All analyses were performed with IBM SPSS Statistics version 23. Data significance as a result of Analysis Of Variance (One-Way ANOVA) and Mann–Whitney repeated measures were tested at the 5% level of probability.

## RESULTS AND DISCUSSION

Many studies have investigated antimicrobial activities of films containing essential oils (Emiroğlu *et al.*, 2010; Djamel *et al.*, 2012; Pires *et al.*, 2013; Yanwong and Threepopnatkul, 2015). It should be pointed out that most of these studies were performed *in vitro* using pure strains as targets. In the current study, we investigated the antimicrobial activity of PEO containing edible soy protein film against *E. coli*,

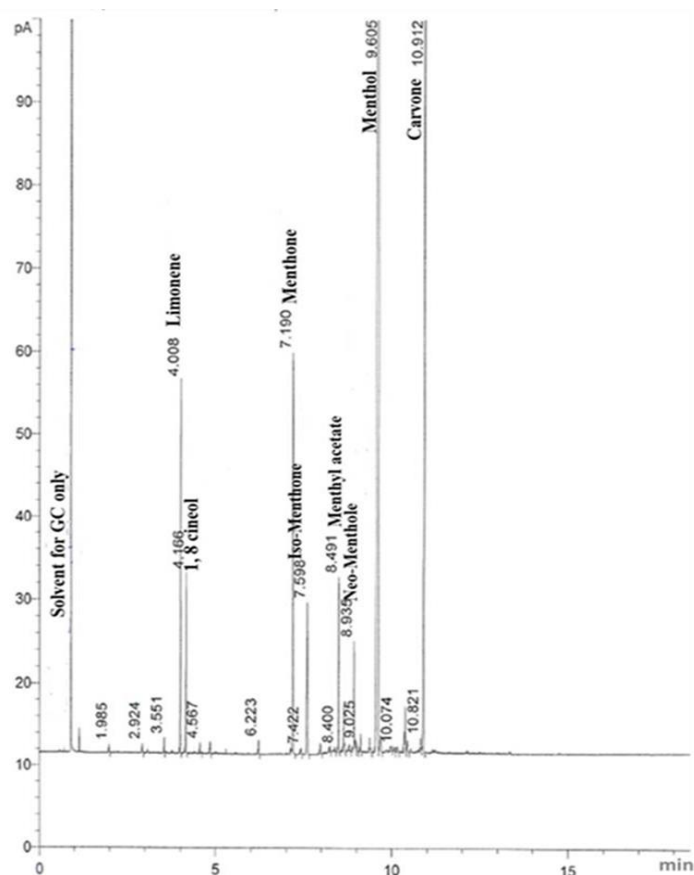
*S. aureus* and *S. enterica* on raw hamburger, as a food model, exposed to the heterogeneous microbial population; furthermore, its ability on extending the shelf life of refrigerated raw hamburger was studied.

### Peppermint Essential Oil Characterization

The percentage of major compounds in PEO was analyzed by using GC-Mass (Figure 1). The main constituents of the samples were menthol (66%), menthone (6.5%), and Carvone (16%). Compared to previous studies, the chemical composition of PEO of this study was different. Masotti *et al.* (2003) and Angioni *et al.* (2006) mentioned that different factors such as; climate, soil composition, plant organ, age and vegetative cycle stage may result in variations in chemical composition of essential oils.

### Physical Properties of ISP Edible Film Containing 1- 3% (v/v) PEO

Table 1 shows the effect of incorporating PEO on the physical properties of ISP-based edible film. Incorporation of 1-3% concentrations of respected essential oil significantly ( $P < 0.05$ ) affected the resulting film thickness, compared to pure ISP based film. On the other hand, no significant difference between PEO was observed in the means of the various concentrations in thickness ( $P > 0.05$ ). The films prepared with 1-3% (v/v) PEO showed lower moisture content than the control film and moisture contents dropped significantly ( $P < 0.05$ ) as PEO concentrations increased. These findings are related to the hydrophobic nature of essential oil, which can affect the ability of the film to retain water. Similarly, WVP decreased noticeably ( $P < 0.05$ ) by addition of PEO into edible film. However, the means of concentrations 2 and 3% were not significantly different. This resulting WVP data is due to the great hydrophobicity



**Figure 1.** Peppermint essential oil GC-MS chromatogram.

**Table 1.** The effect of peppermint essential oil (0-3% v/v) on thickness, moisture content and Water Vapor Permeability (WVP) of ISP edible film.<sup>a</sup>

Treatments	Thickness (mm)	Moisture content (%)	WVP (g mm <sup>-1</sup> kPa h m <sup>2</sup> )
Control	0.078±0.01 <sup>a</sup>	6.39±0.04 <sup>a</sup>	0.054±0.05 <sup>a</sup>
1% PEO <sup>b</sup>	0.136±0.02 <sup>b</sup>	5.49±0.05 <sup>b</sup>	0.41±0.01 <sup>b</sup>
2% PEO	0.143±0.03 <sup>b</sup>	4.72±0.07 <sup>c</sup>	0.37±0.04 <sup>c</sup>
3% PEO	0.157±0.02 <sup>b</sup>	3.91±0.06 <sup>d</sup>	0.34±0.04 <sup>c</sup>

<sup>a</sup> Averages with different letters in the same column differ significantly ( $P < 0.05$ ). <sup>b</sup> PEO: Peppermint Essential Oil.

of film containing essential oil, which has a positive effect on water vapor barrier properties of respected edible film. The capability to control moisture transfer between the food and its surrounding atmosphere is one of the important functions of biodegradable films, especially the protein and polysaccharide based films (Pires *et al.*, 2013). Similar results were obtained by Yao *et al.* (2017) who reported

that the moisture content and WVP of the gelatin-chitosan edible films decreased with increasing *D*-limonene essential oil content compared with the control films. They demonstrated that there was a correlation between WVP values of the edible films and the hydrophilic/hydrophobic ratio of the film component. Essential oil, as a hydrophobic oil phase, uniformly disperses to the edible film, which could prevent water from

transferring through the edible films. As a result, the lower WVP of edible films supplemented with essential oil was achieved.

### Surface Color Measurement

The effects of PEO concentration on  $L^*$ ,  $a^*$ ,  $b^*$ , total color difference ( $\Delta E$ ) and Whiteness Index (WI) of ISP based film are shown in Table 2. The color parameter results of ISP films incorporated with increasing concentration of PEO showed obvious increase in the  $L^*$  (Lightness/darkness) and  $a^*$  (redness/greenness) values compared to those in the control ISP film ( $P < 0.05$ ). However, the  $L^*$  values of the films showed no significant difference between the 1.0% incorporated ISP film and the control. The control sample had an initial yellowness/blueness ( $b^*$ ) value of  $7.4 \pm 0.9$ , and the addition of essential oil [1-3% (v/v)] caused a significant decrease to  $2.90 - 4.08$ . In addition, considerable increment of the Whiteness Index (WI) values were observed compared to the control ( $p < 0.05$ ). These results presented a similar pattern when compared with the  $L$  and  $WI$  values from ISP based edible films with increasing concentrations of allspice, cinnamon and clove essential oils, reported by Du *et al.* (2009). Compared to the control, a noticeable decline of the total color difference ( $\Delta E$ ) value was observed as essential oil contents increased. This result coincides with findings of Estévez *et al.* (2005) who observed that higher levels of

rosemary essential oil (0.03 and 0.06%) caused significant reduction in  $\Delta E$  in comparison with the control samples.

### Tensile Strength (TS) and Elongation at Break (EB)

Table 3 depicts the effect of various concentrations of PEO on  $TS$  and  $EB$  of ISP based edible films. The control film had the highest  $TS$  (17.69 MPa) in comparison with films containing essential oil. This can be attributed to thermal pretreatment and denaturation of the soy protein, which leads to improved intermolecular interaction of disulfide and hydrogen bonds (Choi *et al.*, 2003). Incorporation of essential oil into the ISP based edible film caused a reduction in  $TS$  which significantly changed ( $P < 0.05$ ) when the essential oil concentration increased from 1 to 3% (v/v). In contrast, the  $EB$  values of the film improved ( $P < 0.05$ ) as concentration of essential oil increased [1 to 3% (v/v)]. The enhancement in elongation can probably be due to the plasticizing effect of essential oil in the protein matrix. These results coincide with data of Yanwong *et al.* (2015) who found that addition of increasing concentration of peppermint oil (10-30%) led to a significant decrease in tensile strength, while the elongation value at break showed an obvious increase ( $P < 0.05$ ).

### Antimicrobial Activity of the Edible Film Incorporated with Essential Oil

Inhibition zone diameters produced by ISP based film discs with different

**Table 2.** The effect of peppermint essential oil (0-3% v/v) on  $L^*$ ,  $a^*$ ,  $b^*$ , total color difference ( $\Delta E$ ) and Whiteness Index (WI) of ISP based film.

Treatments	$L^*$	$a^*$	$b^*$	$\Delta E$	WI
Control	$50.11 \pm 2.8^a$	$-6.67 \pm 0.07^a$	$7.4 \pm 0.9^b$	$11.87 \pm 0.67^b$	$48.05 \pm 0.65^a$
PEO <sup>a</sup> 1%	$50.58 \pm 1.72^{ab}$	$-0.2 \pm 0.05^b$	$3.82 \pm 0.11^{ba}$	$2.72 \pm 1.22^a$	$52.48 \pm 1.63^b$
PEO 2%	$54.64 \pm 2.8^{ba}$	$-0.9 \pm 0.1^c$	$2.9 \pm 0.4^a$	$4.86 \pm 0.86^{ba}$	$56.10 \pm 2.81^c$
PEO 3%	$56.92 \pm 1.43^b$	$0.03 \pm 0.00^d$	$4.08 \pm 0.09^{ba}$	$2.57 \pm 0.5^a$	$57.51 \pm 0.00^c$

<sup>a</sup> Peppermint Essential Oil. <sup>abcd</sup> Mean values  $\pm$  Standard deviations in same column for control and EO films with different letters are significantly different at  $P < 0.05$ .

**Table 3.** The effect of peppermint essential oil (0-3% v/v) on Tensile Strength and Elongation at Break of ISP based film.

Treatments	Tensile Strength (MPa)	Elongation at Break (%)
Control	17.69±0.03 <sup>a</sup>	36.33±0.03 <sup>c</sup>
PEO <sup>a</sup> 1%	16.26±0.59 <sup>b</sup>	41.28±0.01 <sup>b</sup>
PEO 2%	11.76±0.21 <sup>c</sup>	57.32±0.02 <sup>ba</sup>
PEO 3%	8.73±0.17 <sup>d</sup>	66.7±0.94 <sup>a</sup>

<sup>a</sup> Peppermint Essential Oil. <sup>abcd</sup> Mean values±Standard deviations in same column for control and EO films with different letters are significantly different at  $P < 0.05$ .

concentrations (0, 1, 2, and 3%) of PEO against 3 test organisms are shown in Table 4. No inhibition zone against test organisms was observed for ISP based edible film with no essential oil. Incorporation of essential oil in ISP based film at the level of more than 2% exhibited a clear inhibition zone. Film containing 2% essential oil was effective against *E. coli* and *S. aureus* strains. However, it did not inhibit *S. enterica* growth. The highest concentration of PEO (3%) resulted in maximum bacterial growth inhibition and was effective against all 3 test strains including *S. enterica*. For *S. aureus*, increasing concentration of essential oil in edible film up to 3% resulted in significantly higher antimicrobial activity ( $P < 0.05$ ). In another study, Emiroğlu *et al.* (2010) determined the inhibitory effect of soy edible films incorporated with thyme and oregano essential oils and noted that *S. aureus* and *E. coli* were significantly inhibited by antimicrobial films, and *S. aureus* was the most susceptible one. Moreover, enhancing essential oil concentrations generally showed an improvement in the growth inhibition levels. Similar results were obtained by Yanwong *et al.* (2015) who reported that gelatin films blended with peppermint essential oils or citronella essential oils were more effective against *S. aureus* (Gram-positive) than *E. coli* (Gram-negative). This is due to the presence of lipopolysaccharides in the outer cell wall of Gram-negative bacteria, which could act as the protective barrier against the phenolic components from essential oils (Yanwong *et al.*, 2015).

### Antimicrobial Effect of Film Containing Essential Oil on Raw Hamburger

According to the results obtained from the preliminary evaluation of physico-mechanical and antimicrobial properties of edible film incorporated with essential oil (study *in vitro*), ISP based edible film with 3% PEO was selected as an adequate formulation for study in a food simulant.

### Effect of Edible Film Containing Essential Oil on Normal Microflora of Uninoculated Raw Hamburger

The raw hamburger samples met the acceptance criteria for microbiological quality and safety based on the Iranian National Standard No. 2304. The total aerobic bacterial count of 2.2 log CFU g<sup>-1</sup> and absence of *E. coli*, *S. aureus* and Salmonella was observed in current study. Therefore, the alterations of total bacterial count in all samples were evaluated, which is an important factor for quality evaluation of raw hamburger. The log CFU g<sup>-1</sup> of total bacterial counts of unwrapped and wrapped samples with or without essential oil during refrigerated storage (at 4°C) is shown in Figure 2. All samples (control, wrapped with and without essential oil) contained about 2.1-2.2 log CFU g<sup>-1</sup> organisms at 0 day. This population in the control and the wrapped samples with no essential oil progressively increased to nearly 8.15 log CFU g<sup>-1</sup> until



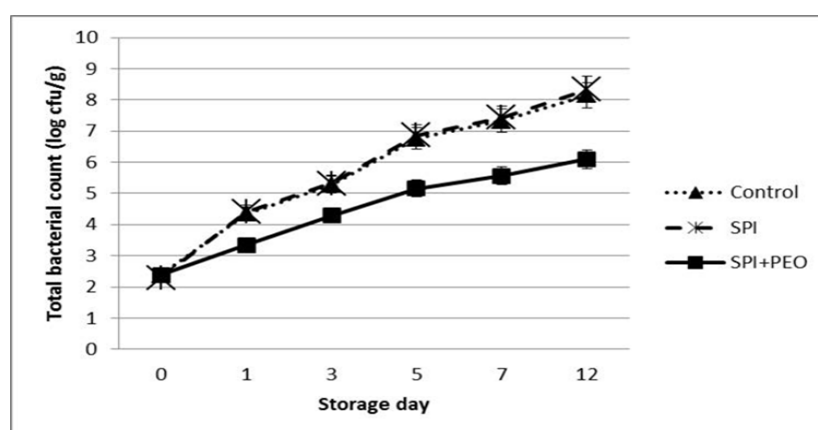
**Table 4.** Inhibition zone diameters (mm<sup>2</sup>) produced by ISP based film discs with different concentrations (0, 1, 2 and 3%) of PEO against 3 test organisms.<sup>a</sup>

Bacteria	PEO (v/v) in bio-edible film (%)	Inhibitory zone (mm <sup>2</sup> )
<i>S. aureus</i>	0	0 <sup>a</sup>
	1	0 <sup>a</sup>
	2	13.23±0.02 <sup>b</sup>
	3	18.34±0.03 <sup>c</sup>
<i>E. coli</i>	0	0 <sup>a</sup>
	1	0 <sup>a</sup>
	2	10.61±0.00 <sup>b</sup>
	3	13.41±0.04 <sup>c</sup>
<i>S. enterica</i>	0	0 <sup>a</sup>
	1	0 <sup>a</sup>
	2	0 <sup>a</sup>
	3	8.61±0.03 <sup>b</sup>

<sup>a</sup> Values are given as the Mean±SD. For each microbial species, different letters in columns indicate a significant difference (P< 0.05).

the end of the refrigerated storage period (7 and 12 days). In contrast, a slight increase of bacterial counts (P >0.05) was observed in wrapped samples with essential oil during days 1, 3, 5, 7 and 12 at 4°C. Significantly lower total count (P< 0.05) was determined in the wrapped samples with film containing essential oil at days 7 (5.56 log CFU g<sup>-1</sup>) and 12 (6.1 log CFU g<sup>-1</sup>) as compared to the control and the wrapped samples with no essential oil, and their microbial count did not exceed the acceptable limit until the end

of storage for 12 days at 4°C. Therefore, ISP based edible film containing 3% PEO were effective in hindering the development of background flora and extending the shelf life of refrigerated raw hamburger for 7 days. Kyriaki *et al.* (2009) reported that 1.5% oregano essential oil incorporated whey protein edible films decreased 3.3 log in total viable count on fresh beef cuts as compared to the control at day 8 of refrigerated storage. This significant difference might be due to the differences in



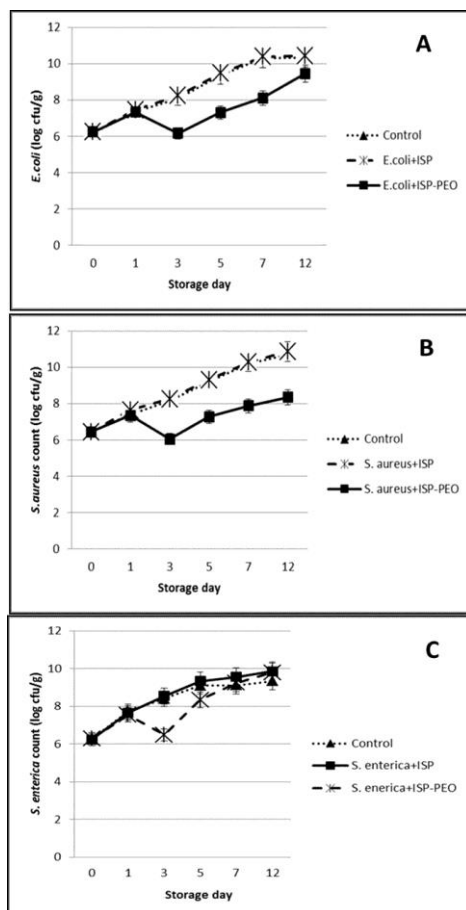
**Figure 2.** Effect of 3% PEO essential oil in ISP film on total bacterial count of hamburger during 12-day storage at 4°C (\* Mean±SD, n= 3, P< 0.05). Points represent mean values (n= 3) and error bars are standard deviations. Control: Unwrapped hamburger; ISP: Wrapped hamburger in ISP Film, ISP-PEO: Hamburger wrapped in ISP-PEO Film.



the meat material used and the complicated structure of ground beef that would inhibit the growth of bacteria in these products.

### Antimicrobial Effect of ISP Edible Film Containing 3% Pepper Mint Essential Oil

Mean of *S. aureus*, *E. coli* and *S. enterica* counts for the unwrapped (control) and wrapped hamburger with and without PEO during 0, 1, 3, 5, 7 and 12 days of storage at 4°C are displayed in Figures 3 (A, B, C). According to the results of Analysis of Variance, there was no significant difference



**Figure 3.** Effect of 3% PEO essential oil in ISP film on bacterial count\* of hamburger during 12 days storage at 4°C: (A) *E. coli*, (B) *S. aureus*, (C) *S. enterica*. Points represent Mean±SD,  $n=3$ ,  $P<0.05$  and error bars are standard deviations. Control: Unwrapped hamburger; ISP: Wrapped hamburger in ISP Film, ISP-PEO: Hamburger wrapped in ISP-PEO Film.

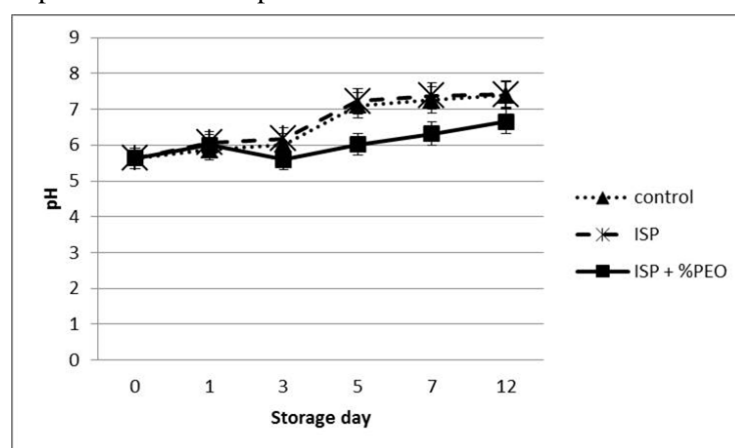
in *S. aureus* count between the control and unwrapped samples with no essential oil, and the initial count of *S. aureus* ( $6.46 \log \text{CFU g}^{-1}$ ) was increased uninterruptedly to 10.68 and  $10.87 \log \text{CFU g}^{-1}$ , respectively, until the end of storage time. The wrapped samples containing essential oil brought about an increase in *S. aureus* count after 1 day of storage followed by a noticeable reduction ( $P<0.05$ ) below the initial count at time zero ( $6.04 \log \text{CFU g}^{-1}$ ) on the 3<sup>rd</sup> day. After that, a slight increase ( $P>0.05$ ) was observed during day 5 and 7 ( $7.28$ - $7.8 \log \text{CFU g}^{-1}$ ) and, finally, bacterial count reached  $8.34 \log \text{CFU g}^{-1}$ , at the 12<sup>th</sup> day of storage. In all samples, the primary count of *E. coli* was  $6.24 \log \text{CFU g}^{-1}$ . Initially, on the first day of storage, the bacterial number increased ( $7.33$ - $7.46 \log \text{CFU g}^{-1}$  with no significant differences among samples. *E. coli* population in the control and the wrapped samples without essential oil continued to increase appreciably and steadily ( $10.34$ -  $10.44 \log \text{CFU g}^{-1}$ ) by day 7, and no significant increment in the bacterial count was observed in day 12. The wrapped samples containing essential oil posed a noticeable decrease in the bacterial count on day 3 ( $P<0.05$ ). However, an increase in the bacterial count was observed by day 7 ( $8.26 \log \text{CFU g}^{-1}$ ), and the medium average counts were lower than those in the control and the wrapped with no essential oil groups. Microbial count in the control sample (0% PEO) was increasingly higher than in the wrapped samples containing essential oil ( $P<0.05$ ). The mean population of *S. enterica*, just after inoculation, was  $6.28 \log \text{CFU g}^{-1}$ . This initial count in the control and the wrapped samples with no essential oil increased rather steadily over the 12-day period, reaching the maximum ranges of  $9.74$ - $9.86 \log \text{CFU g}^{-1}$  at 4°C. In the wrapped samples containing essential oil, respective bacterial count exceeded the initial count in the first day of refrigerated storage, followed by a significant reduction ( $P<0.05$ ) to  $6.48 \log \text{CFU g}^{-1}$  over 3 days. *S. enterica* increased gradually during the remaining storage time

to approximately 8.78 log CFU g<sup>-1</sup>, which was appreciably lower compared to the control and the wrapped samples with no essential oil ( $P < 0.05$ ). In general, although PEO (3%) incorporated film disc had an inhibitory effect on three test bacteria of the current study (Table 3), it did not exhibit considerable inhibitory effect against target bacteria. It was solely able to delay or decelerate the growth of test bacteria over a 12-day refrigerated storage. This outcome is generally in accordance with Emiroğlu *et al.* (2010) study, who reported that ISP films combined with oregano, thyme, or their mixture did not exhibit significant effects on the total viable counts when applied on ground beef patties. This probably points to the fact that the antimicrobial efficacy of edible films depends on factors such as type, concentration of antimicrobials, and the test product properties.

#### Effect of ISP Edible Film Containing 3% PEO on pH Value of Hamburger during Refrigerated Storage

Changes in pH values during storage at 4°C for days 0, 1, 3, 5, 7, 12 are shown in Figure 4. Raw hamburger samples had an initial pH

after 3 days of refrigerated storage period and reached 7.39 on day 12, which paralleled with the increase of total bacterial count as mentioned above. Therefore, their proteolytic activity results in the production of alkaline compounds, which is able to trigger an increase in the pH value. This outcome is supported the study performed by Mexis *et al.* (2009) who reported that the gradual increase of pH value in fish muscle during storage period is due to the accumulation of alkaline compounds derived from microbial action. The wrapped samples with no essential oil exhibited a pH increase after 1 day, and their final pH value reached 7.42 at the end of storage period. It could be attributed to the high pH levels of the ISP edible films (pH, 10.00) as determined in the film solution. Similar result was observed in another study (Emiroğlu *et al.*, 2010) which showed an increase in the pH value of beef patties wrapping with ISP based edible film after 1 day of storage. They were significantly higher compared to the pure samples. In contrast, the wrapped samples containing essential oil exhibited noticeably lower pH value in comparison with the control ( $P < 0.05$ ) and no significant difference ( $P > 0.05$ ) was found in pH values



**Figure 4.** Effect of ISP-PEO on pH value of the hamburger during 12 days storage at 4°C. Points represent Mean±SD, n = 3,  $P < 0.05$  and error bars are standard deviations. Control, ISP, and ISP-PEO as defined in Figure 3.

of 5.63 before wrapping with edible film. A significant increase ( $P < 0.05$ ) in the pH value of the control samples was observed

between days. This can be due to the antimicrobial activity of ISP edible film containing essential oil leading to reduction

of alkaline bacterial metabolite compound (Remya *et al.*, 2016). The pH value of wrapped sample containing essential oil in the day of organoleptic rejection was 6.66 (12<sup>th</sup> day).

#### Effect of ISP Edible Film Containing 3% PEO on Organoleptic Quality of Hamburger

The result of the organoleptic evaluation of hamburger samples (control, wrapped with and without essential oil) during refrigerated storage is presented in Figure 5. The initial overall acceptability score of all samples was 9, which reduced significantly ( $P < 0.05$ ) during storage period in all samples. However, the decrease in the control and the wrapped samples with no essential oil had a steeper slope, and they were rejected organoleptically on the 5<sup>th</sup> day of refrigerated storage when their overall acceptability scores were below 5. This outcome parallels the results obtained from microbial analysis and pH determining of samples during refrigerated storage time in this study. The wrapped hamburgers with essential oil were acceptable until the 12<sup>th</sup> day of storage, thus, extended the shelf life by 7 days in comparison with the control and the wrapped sample with no essential oil. Incorporation of PEO not only did not negatively affect the organoleptic properties of the hamburger, but also the minty taste and the freshness resulting from the slight

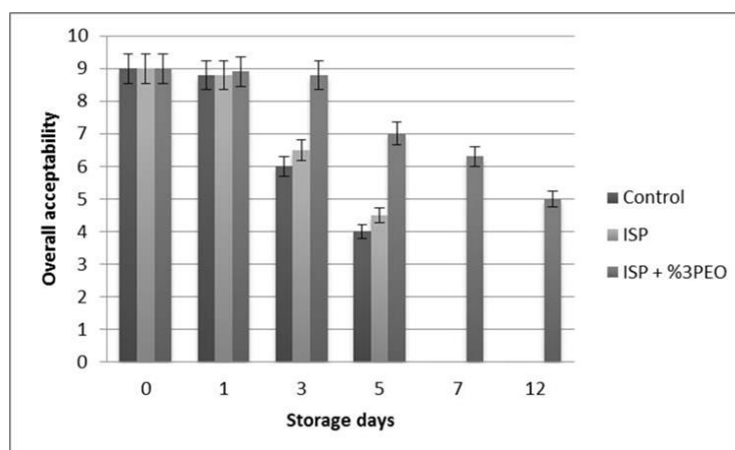
peppermint aroma to the sample was well received by the panelists.

## CONCLUSIONS

Edible films containing plant-derived volatile essential oils pave the way to enhance microbial safety and shelf life of food. In the present study, biodegradable film was successfully prepared from Soy Protein Isolate, and addition of PEO significantly enhanced the antimicrobial and water vapor barrier of ISP based edible film without negatively modifying the physico-mechanical properties of the film. ISP film enriched with 3% PEO could extend the shelf life of raw hamburger stored under refrigerated condition without altering its organoleptic characteristics. Therefore, it has a potential to be applied as ecofriendly and economic active food packaging.

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**Figure 5.** Effect of ISP-PEO on the overall organoleptic score of the hamburger during 12 day storage at 4°C (\* Mean±SD, n= 3, P< 0.05). Error bars are standard deviations. Control, ISP, and ISP-PEO as defined in Figure 3

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## ویژگی های فیزیکی مکانیکی و ضد میکروبی فیلم ایزوله پروتئین سویا حاوی اسانس نعنا فلفلی روی

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## چکیده

محصولات گوشتی بسیار فسادپذیرند و جهت حفظ کیفیتشان، نیازمند محافظتند. اخیراً، فیلمهای خوراکی حاوی اسانس های روغنی، به علت دارا بودن مزیت پوشش فعال ضد میکروبی، توجه زیادی را به خود جلب کرده اند. هدف این مطالعه، ارزیابی ویژگی های فیزیکی مکانیکی و ضد میکروبی فیلم ایزوله پروتئین سویا حاوی اسانس نعنا فلفلی بر عمر مفید و کیفیت همبرگر خام در طی ۱۲ روز در دمای ۴°C می باشد. بدین منظور، فیلم پروتئین سویا حاوی غلظتهای ۱،۲ و ۳ درصد اسانس نعنا فلفلی با روش کاستینگ آماده شد. نتایج نشان داد که افزودن اسانس نعنا فلفلی کاهش و افزایش بارزی را به ترتیب در مقاومت به کشش و ازدیاد طول ایجاد کرد. با افزایش غلظت اسانس، ضخامت و نفوذپذیری نسبت به بخار آب به طور قابل ملاحظه ای افزایش یافت در صورتیکه محتوای رطوبت با کاهش همراه بود. به علاوه، اندیس روشنائی، سفیدی، قرمزی / سبزی و زردی / ابی نیز با افزایش غلظت اسانس افزایش یافتند، درحالیکه تغییرات رنگ تنزل یافت. فیلم حاوی ۳٪ اسانس بیشترین اثر ضد میکروبی را علیه *استافیلوکوکوس اورئوس*، *اشرشیا کولای* و کمترین اثر را علیه *سالمونلا انتریکا* با روش دیسک دیفیوژن نشان داد. آنالیز میکروبی و pH همبرگر خام با پوشش فیلم حاوی اسانس اثر ممانعت کنندگی قابل ملاحظه ای را علیه باکتری های تست روی همبرگر خام نشان نداد، درحالیکه، از رشد بیش از حد مجاز باکتریهای توتال تا اتمام دوره یخچال گذاری ممانعت شد. این فیلم توانست عمر مفید همبرگر خام را تا ۷ روز افزایش دهد. بنابراین این فیلم ضد میکروبی جدید دارای پتانسیل قابل توجهی برای کاربرد به عنوان مواد پوشش دهنده گوشت می باشد.