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ANTIMICROBIAL ACTIVITY OF EGG WHITE PROTEIN-BASED EDIBLE FILMS INCORPORATED WITH THYME AND HOPS LIQUID EXTRACTS ON HAMBURGERS

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ABSTRACT

Antimicrobial activity of egg white protein-based edible films (EWP) incorporated with 5% thyme (TV), 5% hops (HL), and 2.5% thyme + 2.5% hops (TH) liquid extracts was evaluated on hamburgers during refrigerated storage (4 °C). Physical properties such as color and thickness of the films were determined, and according to the results, it was determined that the addition of the extract decreased the *L** value, increased *a** and *b** values, slightly increased the thickness value in the films. The average total viable count, yeast-mold, coliform group bacteria, *Staphylococcus* spp., and *Pseudomonas* spp. numbers of the hamburgers varied between 6.12-8.43 log CFU/g, 2.50-5.46 log CFU/g, 4.87-7.35 log CFU/g, 4.15-6.17 log CFU/g, and 6.10-9.22 log CFU/g, respectively. The groups with hops extract supplements have always had the lowest number of coliforms, indicating that hops extract has higher antimicrobial activity on the coliform group bacteria compared to especially the control group ($p<0.05$). The film application reduced the brightness, and the thyme extract increased the yellowness in the hamburgers. The redness was not affected by storage and treatment factors. While the pH value is very close to each other in the film-packaged groups, it exhibits a constantly increasing slope in the control group.

Abbreviations: C, control group unpackaged with film; EWP, antimicrobial-free film; TV, 5% (v/v) thyme (*Thymus vulgaris* L.) extract added film; HL, 5% (v/v) hops (*Humulus lupulus* L.) extract added film; TH, 2.5% (v/v) thyme extract + 2.5% (v/v) hops extract added film

1.Introduction

Passive packaging techniques, which only have protection functions, have been replaced by new packaging technologies (Delikanli and Ozcan, 2014). Among these technologies, active packaging, which actively protects the product from its environment and adds value to the basic function of packaging, is one of the subjects that researches are concentrated (Suppakul *et al.*, 2003). In general, in active packaging, by maintaining quality an approach based on the relationship between food, packaging material, and environmental atmosphere is applied to extend the shelf life of food, improve food safety, and sensory properties (Cha and Chinnan, 2004). Antimicrobial packaging, which is one of the active packaging types, is a suitable storage method especially for red meat, poultry, and seafood (Suppakul *et al.*, 2003). The basis of the antimicrobial packaging, developed to completely inactivate or limit the growth of existing or reproducible microorganisms in foods, by adding

antimicrobial agents to the packaging material or its environment alone or combination, is a controlled release of the migration of antimicrobial agents. As a result, not only the initial microorganisms are inactivated, but the antimicrobial activity will be longer during the storage and transportation of the product, thus preventing the development of microorganisms that may occur (Cutter, 2002).

There are five basic protein fractions in egg white; ovalbumin, ovotransferrin, lysozyme, ovomucin, and ovomucoid. Ovalbumin, which makes up more than semi of egg white protein by weight, is the just fraction includes free sulfhydryl groups (SH). Other proteins, such as ovotransferrin and lysozyme, include disulphide bonds (S-S) (Mine, 1995). Ovotransferrin is an iron-binding protein. Lysozyme has antimicrobial activity and has been discovered to be effective against Gram-negative bacteria (Dangaran *et al.*, 2009). The film formation mechanism is assumed to include intermolecular and intermolecular S-S bonds. At alkaline pH, S-S bonds are reduced to SH groups, thus simplifying protein dispersal. Heating also opens the protein chains, revealing more hydrophobic and SH groups. During gelation and drying, SH groups are turned into intermolecular and intramolecular S-S covalent cross-links thanks to sulfhydryl-disulfide exchange reactions and oxidation (Mine, 1992; Gennadios *et al.*, 1996). This results in the formation of three-dimensional networks (Lim *et al.*, 2002).

Thymus vulgaris L. is found in the *Lamiaceae* family. Thymus are significant medicinal plants that are known to include antimicrobial substances and are rich in dissimilar active substances such as carvacrol, thymol, γ-terpinene, thymyl methyl ether compounds (Nabavi *et al.*, 2015). Studies have shown that thyme essential oil and extract have powerful antimicrobial (Imelouane *et al.*, 2009; Rota *et al.*, 2008) and antifungal activity (Del Toro-Sánchez *et al.*, 2010; Rasooli and Abyaneh, 2004). Thyme extract can be used as a natural preservative in the preparation of active food packages (Aziz and Almasi, 2018). *Humulus lupulus* L. is found in the *Cannabaceae* family. Between the dissimilar parts of hops, just the female cones and leaves showed antimicrobial properties (Zanoli and Zavatti, 2008; Abram *et al.*, 2015). The antimicrobial activities of multifarious hops extracts are known, as well as the singular hops parts. Two hops bitter acids, humulones (alpha acid) and lupulones (beta acid), showed activity against Gram-positive bacteria such as *Bacillus*, *Clostridium*, *Lactobacillus*, *Listeria*, *Staphylococcus*, *Streptococcus* species (Haas and Barsoumian, 1994; Bhattacharya *et al.*, 2003; Shen *et al.*, 2009; Siragusa *et al.*, 2009; Teuber and Schmalreck, 1973), Gram-negative bacteria such as *Brucella* species and *Helicobacter pylori* (Ohsugi *et al.*, 1997; Shapouri and Rahnema, 2011), and fungi such as *Trichophyton*, *Mucor*, *Fusarium*, *Candida* species (Mizobuchi and Sato, 1985).

In this study, thyme and hops liquid extracts were added alone and combination to edible films based on egg white protein applied to hamburgers. In this way, it is aimed to prolong the shelf life of the product and prevent economic losses with a new active packaging technology by inactivating and/or limiting the growth of microorganisms that may be present in the product or may be occur later in storage.

2. Materials and methods

2.1. Materials

The edible film material used in the research is based on egg white protein and Alfasol brand egg white protein powder was obtained from Kimbiotek Chemical Agents Inc. (Istanbul, Turkey). Thyme (*Thymus vulgaris* L.) liquid extract from antimicrobial agents was obtained according to the method proposed by Xu *et al.* (2008). Another antimicrobial agent, hops (*Humulus lupulus* L.) liquid extract was obtained from Gökçek Şifa Inc. (Istanbul, Turkey).

2.2. Methods

2.2.1. Thyme liquid extract and edible film production

For the production of thyme liquid extract from the antimicrobial substances used in the research, dried thyme was purchased from the herborists in the Konya market, and then ground and ground into powder. 20 g of powdered thyme and 180 ml of distilled water were placed in a flask and kept in a shaking water bath (Wisd, Korea) at 90 ℃ at 150 rpm for 30 minutes. Then incubated in an oven (Nuve EN 120, Turkey) at 37 ℃ for 1 night. Finally, the liquid extract was obtained using Whatman No. 1 filter paper (Xu *et al.*, 2008).

In the production of an edible film based on egg white protein, Gennadios *et al.* (1996) and Kavas (2017) suggested methods were modified and used. For this purpose, 9 g of egg white protein powder, 100 ml of distilled water, and 4.5 ml of glycerol as a plasticizer were homogenized (Wisd HG-15D & HG-15A, Korea) at 700 rpm for 1 minute, and then the pH of the solution was adjusted to 11.25 with 1 N NaOH. Then film solutions were maintained wherein the water bath (Nuve BM 402, Turkey) at 45 ℃ for 20 minutes, and the antimicrobial-free film solution was filtered after cooling to room temperature. Films containing thyme and hops liquid extract were filtered after they were homogenized with a homogenizer (Wisd HG-15D & HG-15A, Korea) at 700 rpm for 1 minute by adding the pre-determined (Karagoz-Emiroglu *et al.*, 2010) 5% (v/v) levels of these substances to the solution. Finally, 15±0.1 g the film solutions were weighed Petri dishes, and were dried wherein the oven (Nuve FN 120, Turkey) at 50 ℃ for 18 hours. The edible films prepared were kept in a vacuum desiccator at room temperature until applied to hamburgers (Figure 1).

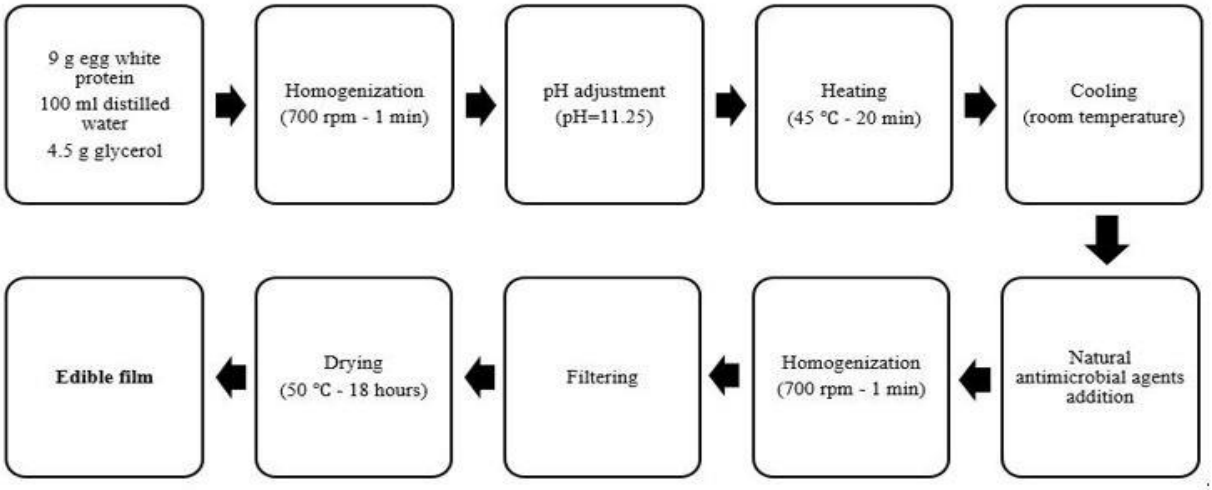

Figure 1. Preparation of edible films

2.2.2. Proximate analysis

Moisture (oven), fat (Soxhlet extraction), protein (Kjeldahl), and ash (ash oven) contents were determined using standard AOAC methods (AOAC, 2000). Moisture (%) was determined by drying 5-10 g of the sample until it reached constant weight at 105 ℃. The fat (%) was determined using the Soxhlet extraction device. Protein (%) was determined according to the Kjeldahl method. The factor 6.25 was used to convert nitrogen to crude protein. Ash (%) was determined by burning 2-2.5 g of the sample until it reached constant weight at 550 ℃.

2.2.3. Physical properties of edible films

The colors of the produced films were determined using a colorimeter device (CR-400, Konica Minolta, Osaka, Japan). The device was calibrated with a white standard plate before the measurement and then *L**, *a**, and *b** values of the films were determined (AMSA, 1991). The

thickness of the produced films was determined using a digital micrometer (Mitutoyo 500-181-30 Digital Caliper, Japan) with a precision of 0.01 mm (Seydim and Sarikus, 2006). Measurements were taken at three random locations of the films.

2.2.4. Preparation of hamburgers

Beef used in the research was obtained from contracted butchers in the Konya market and turned into medium-fat minced meat with a fat content of 15-20%. After adding the additives in the hamburger formulation in certain proportions, they were given the proper shape and film application was carried out immediately. As seen in Figure 2, the films were applied by placing them on the upper and bottom surfaces of the hamburgers. Hamburgers were divided into 5 different treatment groups; control group unpackaged with film (C), antimicrobial-free film (EWP), 5% (v/v) thyme (*Thymus vulgaris* L.) extract added film (TV), 5% (v/v) hops (*Humulus lupulus* L.) extract added film (HL) and to determine the synergistic effect of antimicrobial agents 2.5% (v/v) thyme extract + 2.5% (v/v) hops extract added film (TH). The hamburgers prepared in this way were preserved for 4 ℃ at 7 days and subjected to microbiological, color, and pH analyzes on the 1st, 4th, and 7th days of storage.

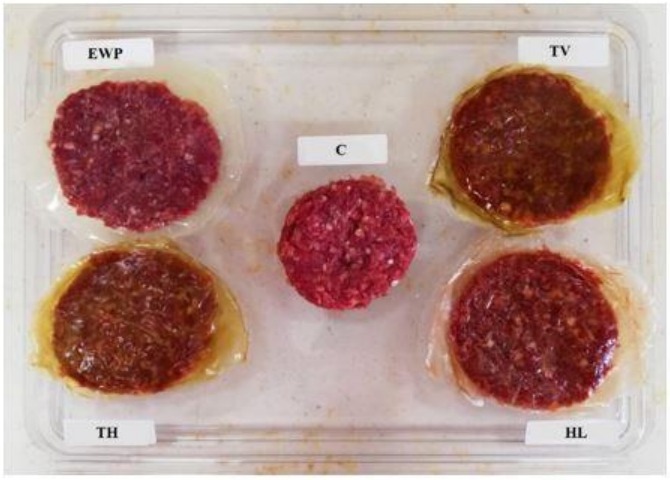

Figure 2. Hamburgers packed with edible films and the control group

Abbreviations: C, control group unpackaged with film; EWP, antimicrobial-free film; TV, 5% (v/v) thyme (*Thymus vulgaris* L.) extract added film; HL, 5% (v/v) hops (*Humulus lupulus* L.) extract added film; TH, 2.5% (v/v) thyme extract + 2.5% (v/v) hops extract added film

2.2.5. Microbiological analysis of hamburgers

In order to perform microbiological analysis, each sample was opened under aseptic conditions and a 10 gram portion was weighed from the center of the hamburgers. In order to make the first dilution, the weighed portion was transferred into the previously sterilized Maximum Recovery Diluent (MRD, Merck) and it was ensured to become homogeneous for 1 minute. Serial dilutions in the range of 10^{-1}-10^{-6} were prepared for all analyses. These prepared dilutions were inoculated on various media by the spread plate method and incubated at certain temperature-time norms. Plate Count Agar (PCA; Merck) medium for total viable count (TVC) and incubation at 37 ℃ for 48 hours, Potato Dextrose Agar (PDA; Merck) medium for yeast-mold and incubation at 28 ℃ for 4-5 days, Violet Red Bile Agar (VRB; Merck) medium for coliforms and incubation at 37 ℃ for 24 hours, Baird Parker Agar (BPA, Merck) + Egg Yolk Tellurite Emulsion medium for *Staphylococcus* spp. and incubation at 37 ℃ for 24 hours, Glutamat Starch Phenol Red Agar (GSP, Merck) + Penicillin G medium for *Pseudomonas* spp. and incubation at 28 ℃ for 3

days conditions were provided. After colony counting, the number of microorganisms as $\log_{10}$colony forming units (CFU)/g is indicated (Halkman, 2005).

2.2.6. Color analysis of hamburgers

The color measurements were performed at days 1, 4, and 7 of refrigerated storage. In this purpose, a colorimeter device (CR-400, Konica Minolta, Osaka, Japan) was used. The device was calibrated with a white standard plate before the measurement and then *L**, *a**, and *b** values of hamburgers were determined (AMSA, 1991).

2.2.7. pH analysis of hamburgers

The pH measurements were performed at days 1, 4, and 7 of refrigerated storage. In this purpose, the pH value of each hamburger was determined by reading from random points with the help of a pH-meter (Testo 205, Germany) (Lambooij *et al.*, 1999).

2.2.8. Statistical analysis

A completely random design was used (two replicates). Data were analyzed using MINITAB software version 16. When a significant ($p<0.05$) main effect was found, differences between means were evaluated using the Tukey's Test.

3. Results and discussions

3.1. Proximate analysis

Moisture, fat, protein, and ash analyzes were conducted to determine the chemical composition of the ground beef used in hamburger making and the results are given in Table 1. In the analyzes performed, the moisture, fat, protein, and ash contents of the ground beef used in making hamburgers was determined as 65.00%, 15.60%, 18.29%, and 1.21%, respectively.

Table 1. Chemical composition of the ground beef (%)

Chemical Composition	%
Moisture	65.00±0.55
Fat	15.60±0.86
Protein	18.29±0.22
Ash	1.21±0.21

Values are means of triplicate samples (±SD)

Gun (2014) was found the amount of moisture, fat, protein, and ash in minced meat samples as 66.19%, 12.67%, 18.70%, and 1.16%, respectively, in his study where he investigated the effect of various dairy by-products on some properties of beef patties. Kececi (2018) was found the amount of moisture, fat, protein, and ash in minced meat samples as 63.99%, 19.02%, 18.10%, and 0.74%, respectively, in his study where he investigated the effect of various vegetable pickle powders on some properties of beef patties. These results and the results we obtained from our study show partial similarity and partial differences. These differences are probably due to the different races, types, ages, and diets of the animals from which the meat was used in the studies.

3.2. Physical properties of edible films

In the study, color and thickness values of edible films based on extracted and non-extracted egg white protein were determined and the results are given in Table 2. When the color values were examined, the brightness-darkness indicator *L** value had the highest value with 87.97 in the edible film based on egg white protein, while the addition of extract caused a decrease in the *L** values of the films ($p<0.05$). The redness-green indicator *a** value had the lowest value with -3.63 in the edible film based on egg white protein, while the addition of extract caused an increase in the *a** values of the films. The liquid extract of hops increased the redness value ($p<0.05$). The yellowness-blue indicator *b** value had the lowest value with 12.61 in the edible film based on egg white

protein, while the addition of extract caused an increase in the *b** values of the films. The liquid extract of thyme increased the yellowness value ($p<0.05$). When the thickness values were examined, it was determined that the thickness of both the extracted and the non-extracted films gave close results, while the addition of the extract increased the thickness in the films somewhat ($p>0.05$).

Table 2. Physical properties of edible films

	EWP	TV	HL	TH
L*	87.97±0.89[a]	74.62±1.42[b]	74.85±2.01[b]	75.90±2.62[b]
a*	-3.63±0.08[c]	0.23±0.86[b]	5.80±1.33[a]	0.25±1.24[b]
b*	12.61±0.96[d]	40.67±0.93[a]	25.47±1.05[c]	36.40±1.97[b]
Thickness (mm)	0.34±0.02[a]	0.35±0.03[a]	0.40±0.09[a]	0.36±0.09[a]

Values are means of triplicate samples (±SD)
[a-d] Means within rows with different superscript letters are significantly different ($p<0.05$)
Abbreviations: EWP, antimicrobial-free film; TV, 5% (v/v) thyme (*Thymus vulgaris* L.) extract added film; HL, 5% (v/v) hops (*Humulus lupulus* L.) extract added film; TH, 2.5% (v/v) thyme extract + 2.5% (v/v) hops extract added film

Kavas *et al.* (2016) examined the application of egg white protein-based films containing orange essential oil on kashar cheese determined the effects of physical, chemical, and antimicrobial properties and reported that the film, which does not contain antimicrobial agents, is brighter and more transparent than films containing essential oil. Similarly, Taqi *et al.* (2011) examined the effects of different olive oil and oleic acid concentrations on the mechanical properties of edible films based on egg white protein and reported that olive oil and oleic acid were added to the film caused a decrease in the films *L** value. They also measured the thickness of the films and they found that the addition of additives caused an increase in the thickness of the films.

3.3. Microbiological analysis of hamburgers

Total viable count (TVC) is a measure to point out the quality of the product that states the non-usability of the product. TVC of the hamburgers packaged with or without edible films during cold storage are presented in Figure 3a. It was determined that the average number of TVC varies between 6.12-8.43 log CFU/g. With increasing time, TVC amounts increased in all treatments ($p<0.05$). The increase in TVC for every treatment during the storage are attached to the first level of microorganism and the level of treatment (Chidanandaiah *et al.*, 2009). Although slightly lower TVC was determined in the hamburgers treated with liquid extracts containing films at day 4 as compared to C and EWP groups ($p<0.05$), no significant differences in TVC was observed between the hamburgers in general ($p>0.05$). This result is similar to the study of Karagoz-Emiroglu *et al.* (2010). Variations in antimicrobial activity between studies may be due to differences in the meat material used. Hamburgers have a complex texture, and it would be not easy to adequately inhibit the growth of microorganisms on hamburgers.

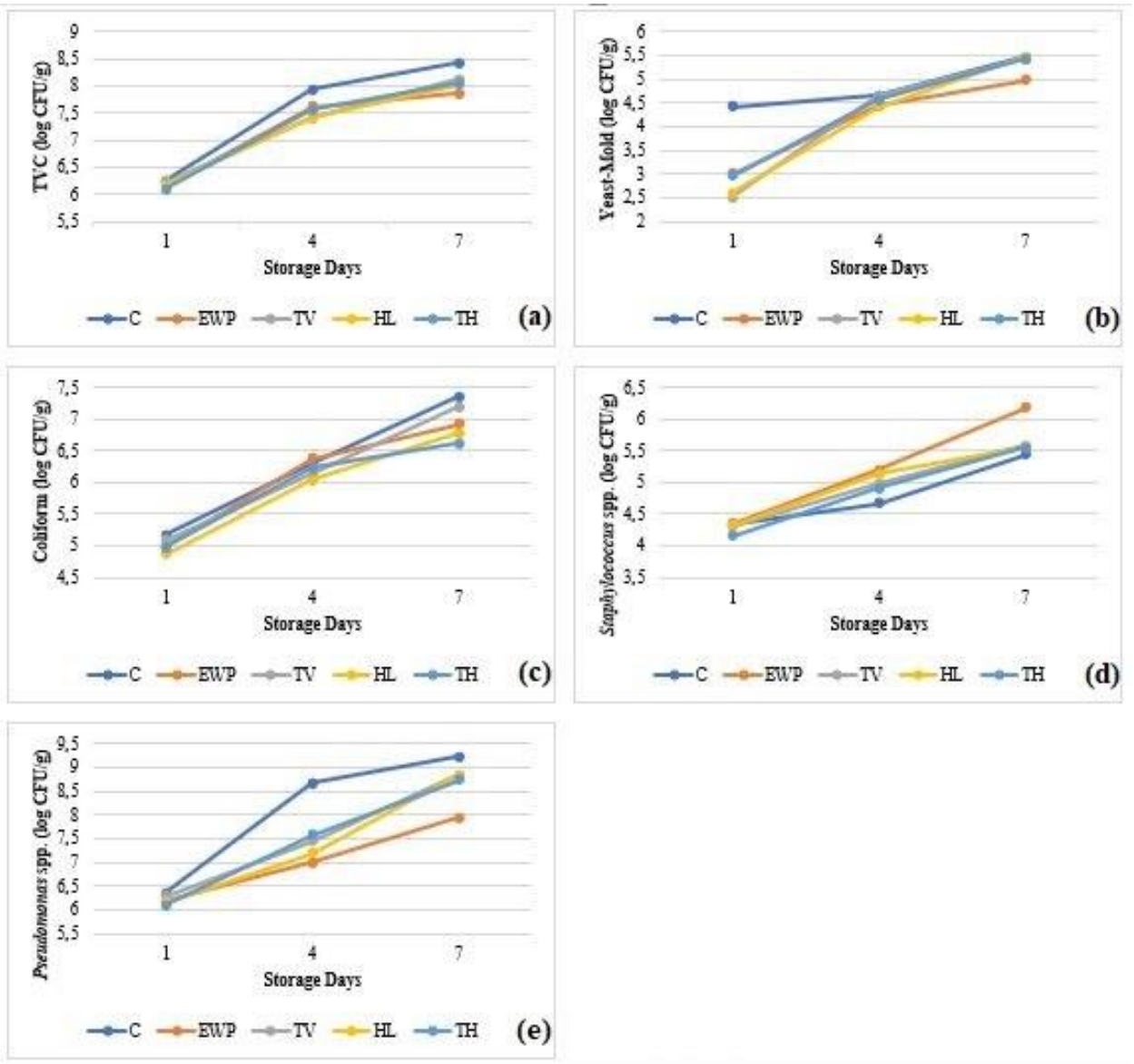

Figure 3. Changes in total viable count (TVC) (a), yeast-mold (b), coliform (c), *Staphylococcus* spp. (d), *Pseudomonas* spp. (e) of different treatment during cold storage (4±1 °C) for 7 days

Abbreviations: C, control group unpackaged with film; EWP, antimicrobial-free film; TV, 5% (v/v) thyme (*Thymus vulgaris* L.) extract added film; HL, 5% (v/v) hops (*Humulus lupulus* L.) extract added film; TH, 2.5% (v/v) thyme extract + 2.5% (v/v) hops extract added film

Yeast-mold counts of the hamburgers packaged with or without edible films during cold storage are presented in Figure 3b. It was determined that the average number of yeast-mold varies between 2.50-5.46 log CFU/g. An increase in yeast-mold counts was observed in parallel with storage ($p<0.05$). The control group has always been the group with the highest yeast-mold count ($p>0.05$). The less count of yeast-mold in liquid extracts treatments can be due to phenolic compounds. Phenolic compounds in the outer membrane of the plant destroy microorganisms, induce the let out of liposaccharides and rise the permeability of the cytoplasmic membrane to ATP. Withdrawal of ATP is concluded in the accomplishment of cellular energy storage and cell death (Burt, 2004).

When coliform group bacteria is mentioned, it is understood that Gram (-), non-spore

forming, rod-shaped bacteria that form acid and gas from lactose within 48 hours at 37 ℃. According to this; *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Citrobacter freundii*, which are members of the *Enterobacteriaceae* family, are defined as coliform group bacteria (Halkman, 2005). Although the consumption of ground raw meat is one of the most common risks of food-borne pathogenic infections, *E. coli* has been identified in many foods all over the world, confirming this risk (Solomakos *et al.*, 2008). Coliform counts of the hamburgers packaged with or without edible films during cold storage are presented in Figure 3c. It was determined that the average number of coliform varies between 4.87-7.35 log CFU/g. Parallel to the storage, an increase in the number of coliforms was observed and the results were found statistically different from each other ($p<0.05$). The groups with hops extract supplements have always had the lowest number of coliforms, indicating that hops extract has higher antimicrobial activity on the coliform group bacteria compared to especially the control group ($p<0.05$). Arsene *et al.* (2015) investigated the antimicrobial and antioxidant activity and phenolic content of hops ethanol extract. The antimicrobial activity was determined by disc diffusion method against several Gram (+) (*Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*) and Gram (-) (*Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas fluorescens*) bacteria. The results showed that hops extract can be used effectively as a plant-based antimicrobial product.

Staphylococcus spp. counts of the hamburgers packaged with or without edible films during cold storage are presented in Figure 3d. It was determined that the average number of *Staphylococcus* spp. varies between 4.15-6.17 log CFU/g. An increase in *Staphylococcus* spp. counts was observed in parallel with storage ($p<0.05$). While the C group had the lowest *Staphylococcus* spp. numbers, the EWP group had the highest *Staphylococcus* spp. numbers ($p>0.05$). It is thought that this situation is due to the fact that *Staphylococcus* spp. are naturally found in the skin and nose flora of humans, and that they were transferred to the films as a result of carelessness during film preparation. Although the addition of extract slightly reduced this situation, the control group gave the lowest *Staphylococcus* spp. Another study in which higher *Staphylococcus* spp. counts were obtained only in the film-coated samples compared to the control and extract-added films was conducted by Jonaidi Jafari *et al.* (2018).

Pseudomonas spp., one of the common meat spoilage bacteria at refrigerated temperatures, counts of the hamburgers packaged with or without edible films during cold storage are presented in Figure 3e. It was determined that the average number of *Pseudomonas* spp. varies between 6.10-9.22 log CFU/g. An increase in *Pseudomonas* spp. counts was observed in parallel with storage except the EWP group ($p<0.05$). The EWP group was the lowest *Pseudomonas* spp. number compared to other groups ($p>0.05$) and reduced the number of *Pseudomonas* spp. by 1.28 log compared to the control group on the last day of storage. Similarly, Bonilla *et al.* (2014) prepared edible films with and without plant essential oils and aimed to control the microbiota of the pork mincemeat with the films they produce. They reported that the films were effective in controlling microbial growth on pork mincemeat, but the inclusion of essential oils did not contribute to antimicrobial activity.

3.4. Color analysis of hamburgers

Alterations in meat color during storage are significant for consumer admission and purchasing decision. It was determined that the brightness-darkness indicator L^* values of hamburgers varied between 34.85-43.98, the redness-green indicator a^* values of hamburgers varied between 9.71-16.09, the yellowness-blue indicator b^* values of hamburgers varied between 3.95-11.94 on average (Table 3). Film application caused a decrease in the brightness of the samples ($p<0.05$). This impact may be thanks to the oxygen barrier property of edible films, which can postpone oxygen diffusion, and

its reaction with myoglobin (Bojorges *et al.*, 2020). The discoloration in meats is extremely associated with myoglobin content in the muscles; beef includes an oxidative muscle type I with a high myoglobin content (Astruc, 2014). The *a** value was not affected by storage and treatment factors, and the difference between treatments was not statistically significant ($p>0.05$), and data demonstrate the efficacy of using edible films with and without antimicrobial agents to protect color unity. Thyme extract increased a yellowness in the samples ($p<0.05$). This result is compatible with the *b** values in the physical properties of edible films. This change may be caused by the presence of carvacrol (yellow color) in the edible films.

Table 3. Analysis results of color and pH values of hamburgers packed with edible films and control group during cold storage (4±1 °C) for 7 days

	Storage Days	Treatments C	EWP	TV	HL	TH
	1	42.81 ± 1.63^{Aa}	38.97 ± 3.73^{Aabc}	36.26 ± 2.31^{Ac}	37.21 ± 2.20^{Abc}	40.63 ± 2.14^{Aab}
L*	4	41.03 ± 3.93^{Aa}	37.62 ± 1.67^{Aab}	37.92 ± 1.93^{Aab}	36.40 ± 2.14^{Ab}	40.44 ± 1.95^{Aab}
	7	43.98 ± 1.62^{Aa}	38.94 ± 2.54^{Abc}	35.16 ± 1.43^{Ac}	40.11 ± 4.17^{Aab}	34.85 ± 2.09^{Bc}
	1	12.18 ± 0.40^{Bb}	16.09 ± 2.38^{Aa}	15.57 ± 2.40^{Aa}	16.06 ± 1.11^{Aa}	10.27 ± 1.60^{Bb}
a*	4	15.12 ± 1.89^{Aa}	14.33 ± 1.21^{ABa}	9.71 ± 2.60^{Bb}	14.17 ± 1.23^{ABa}	10.82 ± 1.21^{ABb}
	7	12.80 ± 0.56^{Ba}	11.31 ± 2.89^{Ba}	12.13 ± 2.06^{ABa}	11.75 ± 2.34^{Ba}	12.77 ± 1.71^{Aa}
	1	7.90 ± 0.46^{Ab}	5.48 ± 1.51^{Ab}	11.94 ± 4.57^{Aa}	7.14 ± 0.62^{Ab}	7.21 ± 0.58^{Ab}
b*	4	7.31 ± 0.98^{ABa}	4.96 ± 0.81^{Ab}	7.76 ± 1.01^{Aa}	6.04 ± 1.42^{Aab}	6.82 ± 1.03^{Aa}
	7	6.64 ± 0.79^{Bab}	4.45 ± 2.04^{Abc}	8.31 ± 1.67^{Aa}	3.95 ± 1.04^{Bc}	7.83 ± 1.48^{Aa}
	1	5.41 ± 0.02^{Cb}	5.58 ± 0.10^{Aab}	5.57 ± 0.10^{ABab}	5.64 ± 0.11^{Aa}	5.69 ± 0.20^{Aa}
pH	4	5.68 ± 0.18^{Ba}	5.49 ± 0.03^{Ab}	5.46 ± 0.06^{Bb}	5.42 ± 0.02^{Bb}	5.46 ± 0.02^{Bb}
	7	6.80 ± 0.07^{Aa}	5.60 ± 0.14^{Ab}	5.67 ± 0.14^{Ab}	5.52 ± 0.06^{Bb}	5.67 ± 0.17^{ABb}

Values are means of duplicate samples (±SD)

[A–C] Means within columns with different superscript letters are significantly different ($p<0.05$)

[a-c] Means within rows with different superscript letters are significantly different ($p<0.05$)

Abbreviations: C, control group unpackaged with film; EWP, antimicrobial-free film; TV, 5% (v/v) thyme (*Thymus vulgaris* L.) extract added film; HL, 5% (v/v) hops (*Humulus lupulus* L.) extract added film; TH, 2.5% (v/v) thyme extract + 2.5% (v/v) hops extract added film

3.5. pH analysis of hamburgers

It was determined that the pH values of hamburgers varied between 5.41-6.80 on average (Table 3). The pH values of the samples covered with films first decreased, then increased, and gave close results to each other ($p>0.05$). While the pH value of the control group was the lowest on the first day, it gradually increased and reached the highest values on the fourth and seventh days ($p<0.05$). Venkatachalam and Lekjing (2020) were coated pork patties with edible films prepared by adding different antimicrobial substances, and they obtained that the pH values of the film coated patties were almost similar while the pH value of the control group gradually increased. The pH increase with storage time may be due to protein denaturation and collection of alkaline by-products such as amines, ammonia and trimethylamine, all produced during amino acid degradation by microbial or autolytic reactions (Lorenzo *et al.*, 2014). During storage, the pH of hamburgers covered with films had slower rate of increased than with control treatment, throughout the storage time. This was probably due to egg white protein, thyme liquid extract,

and hops extract having antimicrobial activity toward various spoilage bacteria, including volatile basic nitrogen producing microorganism (Ehsani *et al.*, 2014).

4. Conclusions

In this study, the effects of edible films based on egg white protein, containing thyme and hops liquid extract, as natural antimicrobial agents, on microbial inactivation of hamburgers with high production and consumption potential from meat products was investigated. Ground beef used in the study has 65.00% moisture, 15.60% fat, 18.29% protein, and 1.21% ash content. Besides, color and thickness values of edible films were measured before hamburgers were packaged. When the color values were examined, it was determined that the addition of the extract decreases the *L** value and increases the *a** and *b** values in the films. When the thickness value were examined, it was observed that the addition of the extract slightly increased the thickness value in the films. According to the results of the microbiology analysis, it was determined that the average total viable count, yeast-mold, coliform group bacteria, *Staphylococcus* spp., and *Pseudomonas* spp. numbers of the samples varied between 6.12-8.43 log CFU/g, 2.50-5.46 log CFU/g, 4.87-7.35 log CFU/g, 4.15-6.17 log CFU/g, and 6.10-9.22 log CFU/g, respectively. The groups with hops extract supplements have always had the lowest number of coliforms, indicating that hops extract has higher antimicrobial activity on the coliform group bacteria compared to especially the control group. According to the results of the color analysis, it was determined that the film application decreased the brightness in the samples, while the thyme extract increased the yellowness in the samples. The redness was not affected by storage and treatment factors. According to the results of the pH analysis, the pH values of the samples covered with films first decreased, then increased, and gave close results. The pH value of the control group increased continuously and reached the highest level among all samples on the last day of storage. It is thought that more sufficient inhibition effects can be achieved with different film raw materials, different antimicrobial agents, and various combinations of them, and it is suggested that it will be beneficial to continue research on this subject.

5. References

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