



Effect of Evaporated Ethyl Pyruvate on Reducing *Salmonella* Enteritidis in Raw Chicken Meat

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ABSTRACT

In this study, the effect of evaporated ethyl pyruvate (EP) was evaluated for the decontamination of *Salmonella* Enteritidis on chicken leg meat as a safe alternative to antimicrobial agent. Also, total aerobic mesophilic bacteria (TAMB), *Enterobacteriaceae*, *Escherichia coli* and yeast-mold counts samples were investigated. Subsequently, the samples were injected with 0, 42, 105 and 420 mg evaporated EP/L air to the paper filter attached to the container cover and stored at +4 °C for 10 days. According to the results, 42 mg EP/L concentration did not cause a significant decrease in *Salmonella* Enteritidis count ($p>0.05$). However, it was determined that 105 and 420 mg EP/L treatments reduced the number of *Salmonella* Enteritidis by more than 1 and 2 log, respectively. EP application also significantly influenced the number of TAMB, *Enterobacteriaceae* and yeast-mold. These results indicate that EP is an effective antimicrobial that could be used to enhance the safety of chicken meat.

INTRODUCTION

The poultry meat has become the second crop after beef and has proven to be an alternative to the closure of the protein opening. Chicken meat is more economical and show lower levels of cholesterol when compared to other poultry meats, so chicken meat consumption has increased (Caki, 2007). Poultry meats are highly perishable to bacterial contaminants due to large amounts of variable nutrients, a high water activity (aw) and a higher final pH limiting the shelf-life of the product (Cantalejo *et al.*, 2016). *Salmonella* sp., *Listeria monocytogenes* and *Campylobacter* sp., which cause diseases in man, can be found on poultry carcasses and in poultry processing plants (Nierop *et al.*, 2005). Mostly, taste and smell modifications haven't occurred in *Salmonella* grown meats.

Salmonella is the most commonly reported cause of foodborne disease among bacterial infections. It is estimated that about 94 million cases of gastroenteritis due to *Salmonella* species occur annually worldwide, leading to 155,000 deaths every year. Among *Salmonella* species, *Salmonella* Enteritidis that cause human nontyphoidal salmonellosis is isolated predominantly from poultry. In recent years, *S. Enteritidis* has been reported as a major causative agent of foodborne gastroenteritis in humans (Asif *et al.*, 2016). The contamination of *Salmonella* sp. may occur throughout the chicken production chain and processing steps. Head pulling and evisceration are considered potential risk factors that contribute to high incidence of *Salmonella* sp. in chicken carcasses (Hungaro *et al.*, 2013).

There are some physical interventions that have been used to reduce the bacterial contamination on chicken carcasses. They include



mainly, water-based and steam treatments, irradiation, ultrasound, high hydrostatic pressure processing and pulsed electric field processing. Amongst these physical methods, water-based and steam treatments have been used frequently for the decontamination of poultry carcasses, and yielded reductions for various bacterial species in the range from 0.9 to 2.1 and 2.3 to 3.8 orders of magnitude, respectively (Loretz *et al.*, 2009).

Changes in the appearance and quality of carcasses, the need for equipment and operator training, and consumer rejection constitute major limitations for the application of physical methods. Also, during slaughter and processing, chemicals such as trisodium phosphate, chlorine-based compounds and organic acids are used in some countries to assist in the reduction of microorganisms in poultry carcasses (Del Río *et al.*, 2007). For example, after treated with trisodium phosphate, chlorine-based compounds, and lactic or acetic acid *Salmonella* contamination has been reduced by 0.6–2.3, 0.9–1.1, and 0.8–2.2 log units, respectively. (Buncic & Sofos, 2011). But, the effectiveness of chemical decontamination methods may remain limited as they may not have access to the microbial pathogens which penetrated inner sites of chicken skin. Gaseous phase and volatile antimicrobials can reach the hidden areas of products and thus, have the potential to eliminate disadvantages of such decontamination processes. Several antimicrobials in the gaseous or vapor phase, including chlorine, ozone, allyl isothiocyanate (AIT), methyl isothiocyanate and essential oils have been used to inactivate pathogens on fresh produce (Durak *et al.*, 2012).

Ethyl pyruvate (EP) is one of the volatile antimicrobials and is a lipophilic pyruvate derivative safer and more stable than pyruvate. It has anti-inflammatory and antioxidative effects. It is currently used as a food additive and flavouring agent known as a therapeutic agent. EP treatment is an emerging non-thermal technology for reducing microbial load on the surface of fresh and processed foods. EP can reach and penetrate the sites that microorganisms can be hidden because of that it is so volatile (Chen *et al.*, 2017; Tornuk & Durak, 2015). EP has antimicrobial properties against microorganisms with clinical relevant resistance (bacteria, fungi, moulds, parasites). Furthermore, EP has shown reduced anti-microbial activities against symbiotic microorganisms such as *Lactobacillus* species (Debebe *et al.*, 2016). EP is classified as GRAS (generally recognized as safe) by the U.S. Food and Drug Administration under certain conditions. However, antimicrobial activity of EP against pathogens

and its antimicrobial activity in broiler chickens have not been previously reported. In the present study, the antimicrobial properties of vaporized EP on *Salmonella* Enteritidis ATCC 13076 and on sensorial and other microbiological parameters such as total aerobic mesophilic bacteria, *Enterobacteriaceae*, *Escherichia coli* and yeast-mold counts in chicken leg meat were investigated.

MATERIALS AND METHODS

Bacterial cultures

Salmonella (*S.*) Enteritidis ATCC 13076 was used for decontamination of chicken leg meat samples in this research. The bacterial culture was provided from Yildiz Technical University Food Engineering Department, Istanbul, Turkey. The bacterial culture was stored on agar slants at 4°C. Overnight cultures were prepared freshly for each experiment by incubation at 37 °C for 16 h in tryptone soya broth (TSB, Oxoid, asingstoke, UK). The inoculum cocktail (1:1000 dilution, 10⁸ colony forming units (CFU)/mL) was prepared by mixing 3 ml bacterial suspension in 3 L of 0.1% peptone water. Fresh activated bacterial cells concentration was approximately adjusted to 4 log CFU/mL for stock inoculum solution (Durak *et al.*, 2012).

Preparation of Meat Samples

Chicken leg meats were purchased from a local market in Kırklareli, Turkey and all samples were swiftly taken to the laboratory. Chicken leg meat samples were contaminated with inoculum culture for 10 minutes in the immersion water as initial numbers 10⁴ CFU/g (Durak *et al.*, 2012) before the experiment. For the experiment, chicken leg meat samples were divided into 4 groups (0 (control), 42, 105 and 420 mg/L vaporized EP) with the amounts of 200 g for each sample.

Application of EP

A 200 g sample of inoculated chicken leg meat was placed in a 1-L closed-lid food container (18.00 cm × 25.50 cm × 9.00 cm, Bora Plastic, Istanbul, Turkey). Application of EP (98% purity; Sigma Aldrich, St. Louis, MO) was performed according to the method described by Durak *et al.* (2012) with slight modifications. To generate EP vapor overtime, 105, 260 and 1,050 µL (equivalent to 42, 105, and 420 mg/L air) of EP was deposited onto Kim Wipes tissues (Kimberly- Clark, Rose well, GA) in the 1-liter food containers. After sealing the containers, the samples



were stored at refrigerator temperature (+4°C) for 10 days. The control sample did not contain EP in the sealed containers. The samples stored at 4°C were tested on days 0, 3, 6, 8, and 10. All EP experiments and analyses of samples were performed in duplicate format.

ANALYSES

Microbiological analyses

Microbiological analysis conducted in this study included the determination of *S. Enteritidis* ATCC 13076, at the same time *Enterobacteriaceae*, *Escherichia (E.) coli*, yeast-mold, and TAMB counts using conventional cultural methods. For this objective, the sample (10g) was transferred to 90 mL 0.1% peptone water (Oxoid, Basingstoke, Hampshire, England) and homogenized with Stomacher Lab-Blender 400 (Seward Medical, London, UK). Appropriate 10-fold dilutions of the samples were prepared in sterile peptone water. Appropriate dilutions were inoculated on petri dishes with Xylose Lysine Deoxycholate (XLD) agar (Merck, Darmstadt, Germany) or Brilliant-green Phenol-red Lactose Sucrose agar (Merck) for enumeration of *S. Enteritidis* ATCC 13076 using spread-plate technique. Then the plates were incubated for 24 h at 37°C. After incubation; some biochemical tests were made to identify *Salmonella* species. Specific colonies were counted following the identification (Mahmoud & Linton, 2008). The enumeration of *Enterobacteriaceae* was performed by Violet Red Bile Dextrose Agar (VRBDA, Oxoid, Basingstoke, Hampshire, England) by using pour plating method and plates were incubated at 37 °C for 24 h according to the standard procedure (TS ISO 21528-2:2012). Numbers of beta-glucuronidase-positive *E. coli* were quantified on Tryptone Bile X-Glucuronide (TBX) Agar (Oxoid), followed by 4 h and subsequent 24 h of incubation at 30°C and 44°C, respectively, according to the ISO 16649-2:2001. Total yeast-mold count was determined in Rose Bengal Chloramphenicol Agar (Oxoid) by using surface plating method and plates were incubated at 25°C for 5-7 days (ISO 21527-2:2008). Total count of aerobic mesophilic bacteria (TAMB) in samples was determined by using pour plate method on plate count agar (Oxoid) and incubating the plates at a temperature of 30°C for 24-48 h. All plates were incubated under aerobic conditions (ISO 4833-2:2013). The results were converted to logarithmic values. The analyses were performed in duplicate and the results were expressed as CFU/g.

Growth Inhibition Level

In addition to the determination of bacterial counts, growth inhibition levels (GIL) of *S. Enteritidis* ATCC 13076 caused by the effect of vaporized EP were calculated using the following equation (Eq. 1) as applied by Sagdic (2003):

$$GIL (\%) = \frac{(P_C - P_T)}{P_C} \times 100 \quad (1)$$

Where P_C and P_T are the microbial populations of the control and EP-treated samples at a certain time, respectively.

Visual Evaluation

Control and EP-treated chicken leg samples after inoculation *S. Enteritidis* ATCC 13076 were visually evaluated during the storage period for color, odor, texture, and overall quality (acceptability) by a semitrained panel of 10 individuals and rated with a 7-point hedonic scale, where 1 represented 'dislike extremely' and 7 represented 'like extremely'. Samples were considered acceptable when their mean scores were above 4 (neither like nor dislike) (Tornuk & Durak, 2015; Durak *et al.*, 2012). The degree of decay in the samples and their color, odor and viscosity properties were verbally explained by the evaluators (Tornuk & Durak, 2015).

Statistical analysis

Analysis of variance (ANOVA) was conducted for each variable measured to investigate the effect of treatments during storage time. After the data were obtained, differences were defined with SPSS 18.0 using Tukey's multiple range tests. Differences were considered significant at $p < 0.01$.

RESULTS

Inactivation of *S. Enteritidis* ATCC 13076 by Vaporized Ethyl Pruvate

The inhibitor effect of ethyl pyruvate application at different concentrations on *S. Enteritidis* throughout the storage period for chicken leg meat samples stored at 4°C are shown in Table 1. The starting population of *S. Enteritidis* ATCC 13076 prior to EP application in chicken leg meat samples was determined as 4.91 log CFU/g. When compared with the control group (0 mg EP/L), it was observed that the lowest EP application (42 mg EP/L) could not provide significant inactivation throughout the storage period ($p > 0.05$). However, 105 and 420 mg EP/L applications provided decreases of 1.00 and 1.92 log CFU/g respectively which resulted in a statistically significant difference ($p < 0.01$) (Figure 1).


Table 1 – Inactivation of *S. Enteritidis* ATCC 13076 on chicken leg meat by various concentrations of vaporized ethyl pyruvate (EP) at 4°C in 10 days¹.

Example	Storage Time (Day)				
	0	3	6	8	10
0 mg EP/L	4.40±0.05 ^a	4.63±0.16 ^a	4.68±0.04 ^a	4.87±0.05 ^a	4.91±0.09 ^a
42 mg EP/L	4.40±0.05 ^a	4.33±0.05 ^a	4.64±0.04 ^a	4.31±0.11 ^a	4.53±0.01 ^a
105 mg EP/L	4.40±0.05 ^a	4.09±0.21 ^{ab}	3.91±0.03 ^b	3.85±0.02 ^b	3.40±0.31 ^b
420 mg EP/L	4.40±0.05 ^a	3.34±0.37 ^b	3.11±0.25 ^c	3.10±0.02 ^c	2.48±0.00 ^c

¹ Values include ± standard deviation.

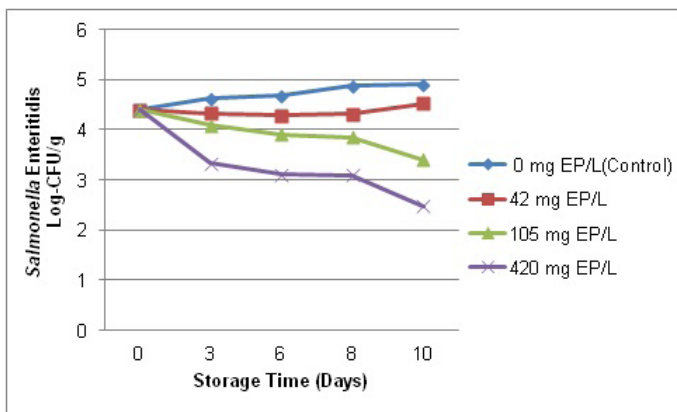
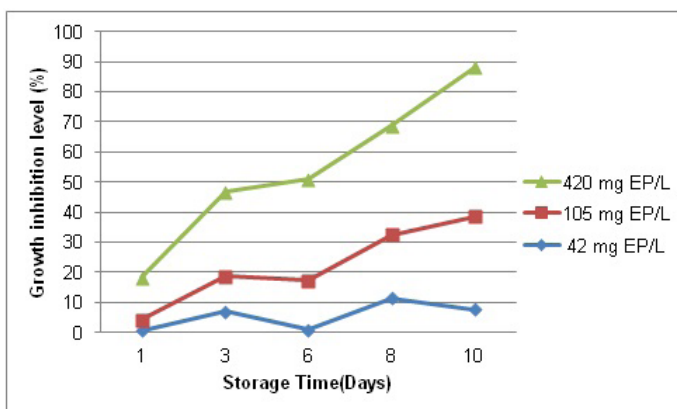
 Values shown in different letters in the same column are statistically different. ($p>0.01$)

Figure 1 – Changes in the number of *S. Enteritidis* of chicken leg meat during storage period.

Figure 5 shows GILs of *S. Enteritidis* ATCC 13076 inoculated to chicken leg meat. The concentration dependence of the inhibition levels can be clearly seen from this figure. At low inoculum levels, there were no remarkable differences between the GILs obtained by 42 mg EP/L treatment. On the other hand, 105 or 420 mg EP/L treatments were notably effective as compared with 42 mg EP/L.

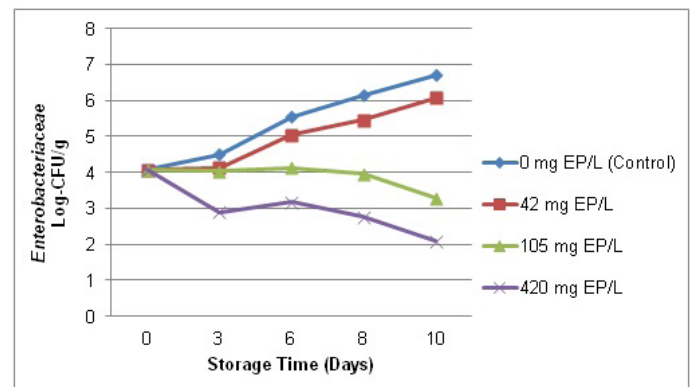

Figure 5 – Growth Inhibition Levels (GIL) of ethyl pyruvate at different concentrations against *S. Enteritidis* on chicken leg meats.

Antimicrobial Activity of EP

Various microbiological analyses (*Enterobacteriaceae*, *E. coli*, TAMB and yeast-mold count) were carried out

on chicken leg samples applied with evaporated ethyl pyruvate and stored at 4°C for 10 days after which the effects of EP application on these microbiological criteria were examined.

When chicken leg meat samples were examined with regard to *E. coli* count, these group bacteria were determined in the control group samples, however *E. coli* development was not observed on EP applied samples. The antimicrobial effect of EP application occurred especially on *Enterobacteriaceae*, TAMB and yeast-mold count. EP application resulted in a statistically significant difference between the samples with regard to *Enterobacteriaceae* count. Whereas *Enterobacteriaceae* count in the control group samples was determined as 6.7 log CFU/g on the 10th day of storage; this value was determined as 6.08; 3.28 and 2.09 log CFU/g for 42, 105 and 420 mg EP/L applied sample groups, respectively. A significant reduction was observed in *Enterobacteriaceae* development depending on the increase in EP concentration in the samples (Figure 2). 105 and 420 mg EP/L applications prevented *Enterobacteriaceae* development at a significant level respectively as 3.42 log CFU/g and 4.61 log CFU/g after 10 days of storage ($p<0.01$).


Figure 2 – Changes in the number of *Enterobacteriaceae* of chicken leg meat during storage period.

A statistically significant inhibition in the TAMB counts was also observed in the samples throughout



the storage period depending on the increase in the EP concentration. Whereas TAMB count was determined as 7.69 log CFU/g on the 10th day of storage for the control group samples; this value was determined as 6.55; 5.19 and 3.76 log CFU/g respectively for 42, 105 and 420 mg EP/L applied sample groups (Figure 3). In comparison with the control group, 42 mg/L EP application resulted in a reduction at a level of 1.14 log CFU/g, 105 mg EP/L application resulted in a reduction at a level of 2.50 log CFU/g and 420 mg EP/L application resulted in a reduction at a level of 3.93 log CFU/g ($p < 0.01$).

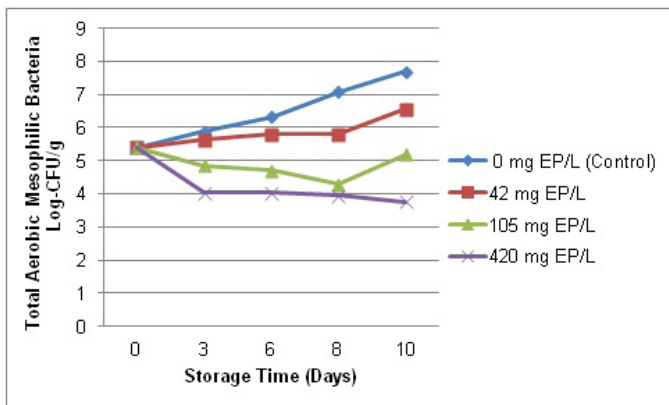


Figure 3 – Changes in the number of total aerobic mesophilic bacteria of chicken leg meat during storage period.

EP application resulted in a significant effect on the yeast-mold count of chicken leg meat samples. Whereas the yeast-mold count of the control group samples was determined as 6.64 log CFU/g on the 10th day of storage; this value on the same day was determined as 5.80; 3.59 and <0.5 log CFU/g for 42, 105 and 420 mg EP/L applied samples respectively (Figure 4). After 10 days of storage, 42, 105 and 420 mg EP/L applications resulted in decreases of 0.84 log CFU/g; 3.05 log CFU/g and <6.14 log CFU/g respectively with the highest inhibitor effect on yeast and mold development ($p < 0.01$).

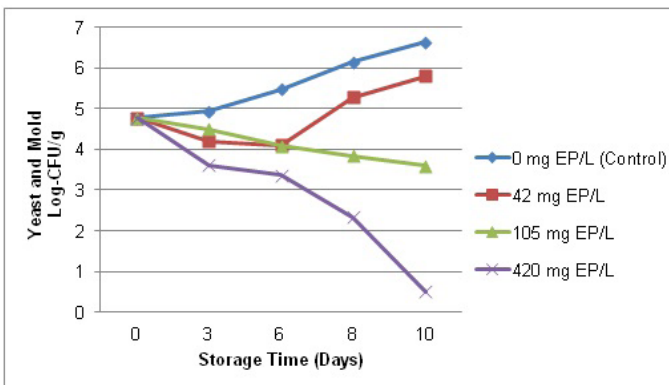


Figure 4 – Changes in the number of yeast-mold of chicken beef samples during storage period

Visual evaluation findings

Chicken leg meat samples were examined visually with regard to color, odor and stickiness throughout the storage period. Evaluators who carried out the visual evaluation put forth that they felt no difference regarding the odor when the samples of 42 and 105 mg EP/L applications were compared with the control group samples. However, strong EP odor was felt throughout the storage period in 420 mg EP/L applied samples. It was stated that EP applied samples were in better condition in comparison with the control group samples regarding the color. The stickiness levels of the samples yielded similar results and it was emphasized that highest EP applied samples (420 mg EP/L) were determined to be more positive with respect to stickiness.

DISCUSSION

The decontamination effects on *S. Enteritidis* ATCC 13076 of EP application evaporated at different concentrations in chicken leg meat samples stored at +4°C were examined in this study. In addition, the effects of this application on various microbial and sensory quality properties of the samples were also examined.

The effectiveness of antimicrobials in the vapor phase generally depend on different factors (Nadarajah *et al.*, 2005; Obaidat & Frank, 2009; Durak *et al.*, 2012). The inhibitor effect of evaporated EP on *S. Enteritidis* increased depending on EP concentration and storage time. Whereas the lowest concentration of 42 mg EP/L evaporated EP application did not result in a significant inhibition effect on *S. Enteritidis* in chicken leg meat samples; it was observed that evaporated EP applications applied at concentrations of 105 and 420 mg EP/L decreased *S. Enteritidis* population in chicken leg meat samples faster and continuously throughout the storage period (Figure 1 and 2). Whereas 105 mg EP/L application resulted in a decrease of 1 log in the samples at the end of the 10th day, EP applied at a concentration of 420 mg/L resulted in a decrease of approximately 1.92 log (Table 1). Increase of EP concentration significantly affects *S. Enteritidis* inhibition as well. It is considered that EP application above 105 mg EP/L shall provide significant *S. Enteritidis* inhibition and that it will be able to prevent low levels of contaminations or recontaminations completely.

Microbial inhibition increases with increasing EP concentration and the shelf life of the samples increases. Durak *et al.* (2012) carried out a study examining *E. coli*



O157:H7 decontamination in green onion and spinach samples with EP application as a result of which it was put forth that the lowest EP concentration (42 mg EP/L) resulted in a reduction of 1.7 in green onion and a reduction of 0.9 log CFU/g in spinach at the end of 7 days storage at 4°C. Researchers observed that under the same conditions, the highest concentration (420 mg EP/L) resulted in a reduction of above 4.7 in green onion and a reduction of 4.3 log CFU/g in spinach. It was emphasized as a result of the study that this application can be used for improving the freshness and shelf life of the product and for developing an alternative preservation method.

Temperature is among the most important factors with impact on the effectiveness of antimicrobials in the vapor phase and this is indicated in the studies carried out (Obaidat & Frank, 2009; Durak *et al.*, 2012). Even though significant inhibition ($p < 0.01$) was attained in samples applied with 105 and 420 mg EP/L, results of studies carried out with vegetables (Durak *et al.*, 2012; Tornuk & Durak, 2015) were below the determined levels. It is thought that quite low (+4°C) storage temperature and the physical structure of the chicken leg meat samples have been effective on obtaining this result. The effectiveness of EP vapor application also modifies with changes in factors such as container composition and surface voids (Durak *et al.*, 2012).

There are studies on *Salmonella* inactivation in foods. It has been suggested that plant hydrosols and especially thyme hydrosol have inhibitor effects on *S. Typhimurium* and that they yield a reduction of above 1 log CFU/g in fruits (Tornuk *et al.*, 2011). 5 mg/L chlorine dioxide gas application for 2 minutes on lettuce resulted in a decrease of 1.5 log in *S. enterica* count (Mahmoud & Linton, 2008). All living cells (5×10^3 CFU) were eliminated in a study carried out for examining the inhibition of *Salmonella* Enteritidis in raw chicken meat with carvacrol vapor (Burt *et al.*, 2007). Spray washing of broiler carcasses with Electrolyzed Oxidated (EO) or Sodium Hypochlorite solution (HOCL) resulted in a decrease in *Salmonella* level by 1.2 and 1.5 log respectively (Northcutt *et al.*, 2007). This new method that was applied for the first time on chicken leg meat samples provides inhibition at the same level or even at a greater level in comparison with the different applications suggested for *Salmonella* decontamination chicken meat and vegetables. In addition, ease of application is the greatest advantage of this method.

In our study, *Enterobacteriaceae*, *E. coli*, yeast-mold and TAMB counts of chicken leg meat samples

were determined along with *S. Enteritidis* in addition to examining the effects of EP application on the microbiological quality of the samples. Evaporated EP applied at a concentration of 420 mg EP/L resulted in a decrease by about 4.6 log in the *Enterobacteriaceae* count of the samples at the end of the 10th day. Whereas *E. coli* was determined in non-EP applied (control group) samples throughout the storage period, this bacteria could not be determined in samples subject to even the lowest EP concentration (42 mg EP/L). This indicates that *E. coli* is quite sensitive to EP application and that evaporated EP may be applied as a quite effective method in comparison with studies carried out for *E. coli* decontamination (Nadarajah *et al.*, 2005; Han *et al.*, 2000; Obaidat & Frank 2009; Vurma *et al.*, 2009; Sagdic *et al.*, 2013).

It has been observed in our study that the yeast-mold load decreased at a statistically significant level based on EP concentration. EP applied at a concentration of 420 mg EP/L decreased all yeast-mold population below the detection limit (<0.5 log) at the end of the 10th day. EP application provided inhibition at levels of 1.14, 2.5 and 3.93 log from the lowest application level towards the highest level in aerobic colony count at the end of a 10 day storage period. It was observed that the increase in EP in our study provided a statistically significant decrease in the TAMB count of chicken leg meat samples.

There are many studies carried out for examining the effects of antimicrobial effects of vaporizable chemicals on various vegetables (Mahmoud & Linton, 2008; Obaidat & Frank, 2009; Bozkurt *et al.*, 2015). For example, Burt *et al.* (2007) carried out a study in which they managed to remove *S. Enteritidis* in raw meat by applying carvacrol vapor. It was determined during these studies that the chemicals applied in vapor phase have statistically significant inhibitor effect on microorganisms. Being subjected to chlorine vapor for long periods of time may result in skin and respiratory tract irritation and chlorine dioxide may explode at high densities (Beuchat, 1998). Allyl isothiocyanate (AIT) is a strong skin and mucosa membrane irritant (Gosselin *et al.*, 1982) and ozone gas is a strong oxidizer, in addition, it may also result in psychological disorders and corrosion on metal surfaces. Ozone generation technologies are also generally complicated and are expensive for many practical applications (Durak *et al.*, 2012). Consideration for sensory quality of chicken leg meat samples within EP treatments, visual properties of the control and EP-treated samples were examined and no differences were observed regarding the odor



or visual change in the samples of 42 and 105 mg EP/L applications. Increasing EP concentration throughout the storage period in 420 mg EP/L applied samples were determined to be lightly positive with respect to stnki. In the previous studies, EP-treated baby spinach and fresh parsley samples had been reported to have generally lower sensorial attributes than those of the control samples after storage (Tornuk & Durak, 2015, Durak *et al.* 2012). In this study EP concentrations also decreased clearly over 7 days of storage at 4°C to <5 mg/L in the containers. Similarly, Durak et al. (2012) reported that only trace of concentrations of EP were detectable at the end of the experiment, and thus, the efficacy of EP vapor treatments may vary with changes in such factors as the container composition or surface area.

It was determined as a result of the study that evaporated EP provides reduction in *S. Enteritidis* count as well as other microbial count in chicken leg meat and that the most ideal application is 105 mg EP/L. In addition to being the first known study examining the antimicrobial activity of EP on *S. Enteritidis*, this study also supports the EP application as an effective decontamination method. Nevertheless, impact mechanism of the EP has not been fully understood yet and this has been expressed in some studies (Durak *et al.*, 2012). It is thought that evaporated EP which is a new application has significant antimicrobial effect, that can be used easily in many foods with high microbial contamination risks, that it may be used as an alternative to storage methods such as modified atmosphere and that it can be applied for the decontamination of carcasses at the slaughter house following the cutting.

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