RESEARCH ARTICLE

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The utilization of vaporized ethyl pyruvate for decontamination of lettuce from *E. coli* O157:H7

Gulsum Ucak Ozkaya[1]

[1] Scientific Research Projects Coordination Unit, Mimar Sinan Art Fine University, Besiktas, Türkiye

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Abstract

The objective of this study was to utilize vaporized ethyl pyruvate (EP) as a means to enhance the safety of lettuce for human consumption. For this purpose, the antimicrobial activity of EP was evaluated on lettuce dipping-inoculated with *Escherichia coli* O157:H7 ATCC 25150. Inoculated samples for antimicrobial analysis and non-inoculated samples for organoleptic analysis (color and sensorial analysis) were treated with 0, 42, 105, and 420 ppm EP and then stored at 4 °C for 7 days and 10 °C for 5 days. Following a storage period of 7 days at a temperature of 4 °C, it was observed that the EP concentrations of 42, 105, and 420 ppm resulted in reductions of 0.8, 1.5, and 3.4 log, respectively, in the population of *E. coli* O157:H7 on lettuce. After a period of 5 days at a temperature of 10 °C, the presence of *E. coli* O157:H7 was observed to decrease by 1.3, 2.1, and 2.2 log. This reduction in bacterial count was attributed to the application of 42, 105, and 420 ppm of EP, respectively. In conclusion, based on the evaluation of organoleptic and color properties, it is suggested that the treatment involving a concentration of 42 ppm EP at 10 °C for 3 days can be a viable non-thermal method for effectively inhibiting bacterial growth.

Keywords: Vaporized ethyl pyruvate, *E. coli* O157:H7, Decontamination, Lettuce, Food safety

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Corresponding Author:
Gulsum Ucak Ozkaya
E-mail: gulsum.ucak.ozkaya@msgsu.edu.tr

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INTRODUCTION

Lettuce (*Lactuca sativa* L.), a member of the cabbage family, is a significant fresh vegetable, and its leaves are widely seen in salads and sandwiches (Mou, 2008, 2009). Lettuce is characterized by its low caloric, fat, and sodium content, while also being a source of dietary fiber, iron, folate, and vitamin C. In addition, lettuce is a notable provider of various bioactive compounds. Lettuce has been found to contain bioactive chemicals that exhibit anti-inflammatory, cholesterol-lowering, and anti-diabetic properties both *in vitro* and *in vivo*. (Kim et al., 2016). Therefore, lettuce may substantially contribute to increasing the nutritional content of diets (Kenny & O'Beirne, 2009). Unfortunately, vegetables are susceptible to infection by pathogenic bacteria, including *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Staphylococcus aureus*, throughout different stages of production, encompassing harvest, storage, and transportation. (Abadias et al., 2008). It is advisable for customers to engage in the practice of washing fresh vegetables and fruits as a precautionary measure against potential microbial contamination resulting from the presence of pathogens. Although the act of washing fresh vegetables under running tap water effectively eliminates dirt and other particles, it does not completely eliminate surface bacteria. Tap water has little or no impact on microorganisms found in fresh vegetables at 10^3-10^9 CFU/g (Koseki et al., 2001).

The market for minimally processed vegetables (MPV) has shown significant growth in recent years due to the heightened consumer awareness. The fresh-cut produce industry frequently uses chlorine solutions as a prevalent technique for mitigating the microbial load on vegetables (Tirpanalan et al., 2011). Nevertheless, the significance of discovering novel decontamination technologies is amplified by public health apprehensions regarding the potential development of trihalomethanes, a known carcinogen, as well as legislative restrictions on chlorine usage (Millan-Sango et al., 2016). Researchers have investigated three distinct categories of alternatives to chlorine disinfection. The three categories of sanitization methods include chemical sanitizers such as chlorine dioxide, hydrogen peroxide, and ozone; natural antimicrobials such as organic acids, plant extracts, and protective cultures; and physical decontamination methods such as irradiation, ultraviolet (UV) treatment, and electrolyzed water (Meireles et al., 2016).

Therein, we utilized ethyl pyruvate (EP), a straightforward derivative of pyruvic acid, as a means to eliminate *E. coli* O157:H7 contamination on lettuce. According to the evaluation conducted by the United States Food and Drug Administration (FDA), EP has been categorized as being safe for consumption in food products. A research group first reported that vaporized EP could be used to decontaminate green onion and baby spinach (Durak et al., 2012) followed by parsley decontamination by Tornuk and Durak (2015), control of postharvest quality and fungal damage of strawberry and cherry fruits by Bozkurt et al. (2016), reduction of *Salmonella* Enteritidis in raw chicken meat by Çetin et al. (2019), inactivation of *Listeria monocytogenes* on sausage surface by Cetin et al. (2019) and recently EP treatment against *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on cherry tomatoes by Ucak Ozkaya et al. (2021). This study aimed to examine the efficacy of vaporized EP decontamination in reducing the presence of *E. coli* O157:H7 on lettuce samples.

MATERIALS AND METHODS

Materials

Lettuce was obtained from a local grocery shop in Istanbul and kept at 4 °C prior to use. The strain *E. coli* O157:H7 ATCC, which was acquired from Acibadem University, was employed in order to assess the antibacterial efficacy of vaporized ethyl pyruvate on lettuce. The stock cultures, maintained at -80 °C with 15% glycerol, were streaked onto Nutrient agar (Merck, Darmstadt, Germany) and incubated at 37 °C overnight. Following this, a single colony was cultured in Nutrient broth (Merck, Darmstadt, Germany) and subsequently incubated at a temperature of 37 °C for a period of 24 h. The initial bacterial inoculum concentration for the dipping inoculation technique to introduce contamination to lettuce was approximately 10.4 log CFU/mL for *E. coli* O157:H7.

Preparation and inoculation of lettuce

Prior to conducting tests for EP treatment, the initial verification of the absence of *E. coli* O157:H7 in the samples was performed. Initial washing with tap water for 10 min was carried out to remove unwanted residues and reduce native bacteria. Following that, the samples were subjected to a deionized water rinse and then dried within a biosafety cabinet at ambient temperature for a duration of 30 min, while being exposed to ultraviolet (UV) treatment. The lettuce samples that were contaminated with *E. coli* O157:H7 were subjected to a drying process at ambient temperature for a period of 2 h subsequent to the inoculation procedure.

Application of vaporized ethyl pyruvate

Lettuce samples that were purposely contaminated with *E. coli* O157:H7 were subjected to treatment using vaporized ethyl pyruvate (EP, 98% purity; Sigma-Aldrich, St. Louis, United States). Specifically, the experiment involved the placement of three lettuce leaves that had been inoculated with microorganisms. Each lettuce leaf was individually placed in a food container with a closed lid. The food container had dimensions of 18.00 cm × 25.50 cm × 9.00 cm and was manufactured by Bora Plastic in Istanbul, Turkey. To maintain humidity, a sponge soaked in 20 mL of deionized water was included in the container. Additionally, Kim Wipes tissues from Kimberly-Clark in Rosewell, GA were used. These tissues were treated with different amounts of EP, specifically 105, 260, and 1.050 µL, which corresponded to concentrations of 42, 105, and 420 ppm, respectively. Samples containing microorganisms and the Kim Wipes tissues added EP were put into the container. Following the closure of the container, EP-treated samples with microorganisms and control samples were subjected to storage conditions of 4 °C for a duration of 7 days, and stored at 10 °C for a period of 5 days.

Microbiological analysis

The spread plate technique was utilized to conduct microbiological analyses at various time intervals during storage. Specifically, analyses were conducted on 0, 1, 3, 5, and 7 days of storage at 4 °C, and on 0, 1, 3, and 5 days of storage at 10 °C. The samples with bacteria, both control and EP-treated, were enumerated for *E. coli* O157:H7 using Sorbitol MacConkey agar (Merck, Darmstadt, Germany). The homogenization of samples was conducted using a Stomacher device (MiniMix 100, Interscience, St. Nom, France) for a duration of 2 min in sterile 0.1% peptone water (1:2, w/v). Subsequently, 1 mL of the resultant mixture was subjected to serial dilution, with each dilution being added to test tubes containing 9 mL of sterile peptone water. The plates were prepared by applying suitable dilutions, followed by incubation at a temperature of 37 °C for a duration of 24 h. After the incubation period, the colonies were counted and expressed as logarithm of colony forming units per gram

(log CFU/g).

Determination of inhibition level

The assessment of growth inhibition levels induced by various concentrations of ethyl pyruvate on *E. coli* O157:H7 was conducted utilizing Equation 1, as reported by Sagdic (2003):

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

The variables and represent the microbial populations of control and EP-treated samples, respectively, at a specific time.

Color analysis

The color of non-inoculated, EP-treated samples and control samples was measured using a colorimeter (Konica Minolta CR-400, Osaka, Japan) at 4 °C on days 0, 1, 3, 5, and 7, and at 10 °C on days 0, 1, 3, and 5. The luminosity value (L^*), chromaticity on the green to red axis (a^*), and chromaticity on the blue to yellow axis (b^*) were measured in triplicate, and the average values were reported.

Sensory assessment

The sensory evaluation was carried out on control (no EP treatment) and EP-treated (42, 105, and 420 ppm) lettuce samples without microorganisms. The samples marked with three-digit numbers were tested on days 0, 1, 3, 5, and 7 at 4 °C and days 0, 1, 3, and 5 at 10 °C. The 20 panelists conducted a simultaneous evaluation of the samples, scoring them based on color, odor, texture, and overall quality.

Statistical analysis

Statistical analysis was conducted using JMP statistical software (version 9.0, 2010, SAS Institute, Cary, NC) after performing all experiments three times on dependent samples. The study employed a two-way analysis of variance (ANOVA) and Tukey's multiple comparison test to examine the disparities in bacterial populations, color, and sensory attributes between lettuces treated with EP and a control group. The data in the tables were presented in the form of mean values accompanied by their corresponding standard deviations. Statistical significance was determined at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

EP-treatment based inactivation of *E. coli* O157:H7 on lettuce

The evaporative nature of the EP facilitates its swift transfer to the stored materials. This particular capability confers a benefit in the elimination of pathogenic bacteria. In the present study, three different EP concentrations, two different storage temperatures, and seven days of storage time were used. Lettuce leaves contaminated with *E. coli* O157:H7 were treated with ethyl pyruvate in a vaporized form. The treated leaves were then stored at 4 ºC for a duration of 7 days and at 10 ºC for a duration of 5 days. Table 1 displays the outcomes of microbial count pertaining to *E. coli* O157:H7 when subjected to EP treatment. The initial population level of *E. coli* O157:H7 that adhered to the lettuce samples was estimated to be approximately 10.4 log CFU/g. The application of EP at concentrations of 42 and 105 ppm did not yield a statistically significant impact on the decontamination of the samples ($p>0.05$). Nevertheless, the inhibition level of the 420 ppm EP treatment on lettuce leaves after one day of storage at 4 °C was found to be 1.2 log CFU/g ($p<0.05$). On days 3 and 5, the reduction amount of 420 ppm EP was 2 and 3.3 log CFU/g, respectively. On the 7th day of storage, all EP concentrations exhibited a substantial reduction in comparison to the control samples ($p<0.05$). However, the most effective EP concentration was determined to be 420 ppm during the storage period at 4 ºC.

On the first day at 10 ºC, the concentration of EP at 420 ppm led to a reduction of 0.8 log in comparison to the control samples. The reduction levels of 42, 105, and 420 ppm EP were 0.9, 1.0, and 1.7 log on the 3rd day of storage, respectively ($p<0.05$). Following a storage period of 5 days, the samples exhibited a notable decrease in the presence of *E. coli* O157:H7. Specifically, the log reductions for *E. coli* O157:H7 were 1.3, 2.1, and 2.2 when treated with EP concentrations of 42, 105, and 420 ppm, respectively. These findings suggest that the application of EP at varying concentrations effectively deactivated the bacteria in the samples compared to the control group. However, no statistically meaningful difference was identified between them on the 5th day at 10 ºC ($p>0.05$). The day when all concentrations are most effective in the inactivation of *E. coli* O157:H7 was found to be the 5th day.

The inactivation of *E. coli* O157:H7 on lettuce was effectively achieved through the application of EP concentrations at 4 °C and 10 °C. Nevertheless, the antimicrobial effect of EP was greater at 4 °C. Several research studies have reported that 10 °C exhibits greater efficacy in terms of bacterial inactivation (Durak et al., 2012; Ucak Ozkaya et al., 2021). While the dipping method was used for inoculation in the present study, the study conducted by Ucak Ozkaya et al. (2021) involved the attachment of bacteria onto the surface of tomato samples through the utilization of the spot inoculation technique. This means that lowering the bacterial density in a specific region could potentially be a more feasible task. Nonetheless, another study indicated that the inoculation method did not significantly affect the effectiveness of UV application on the reduction of bacteria (Guo et al., 2019). In addition, the initial

number of microorganisms attached to the lettuce samples was high. A study on this subject indicates that the effectiveness of the antimicrobial agent employed may be influenced by the concentration of inoculation (Tornuk & Durak, 2015).

Various decontamination methods have been employed to prolong the shelf life of products and eliminate pathogenic bacteria present in fresh fruits and vegetables. Of these, essential oils (Rossi et al., 2019), allyl isothiocyanate (Guo et al., 2018), and ozone (Aday & Caner, 2014) were the most commonly used vaporized antimicrobials. Numerous research endeavors have been undertaken to examine the effects of EP on the eradication of pathogenic bacteria present in unprocessed vegetables and fruit (Bozkurt et al., 2016; Durak et al., 2012; Tornuk & Durak, 2015). Durak et al. (2012) conducted an investigation to evaluate the antimicrobial efficacy of EP in the process of decontaminating green onions and spinach contaminated with *E. coli* O157:H7. The EP treatment demonstrated efficacy in achieving a reduction of greater than 4.7 log in the population of *E. coli* O157:H7 on green onions following the completion of the storage period. These findings were determined to be consistent with our own results. The study conducted by Tornuk and Durak (2015) yielded similar findings, as they utilized EP treatment on fresh parsley to deactivate *S. aureus* and *E. coli* O157:H7.

GILs of *E. coli* O157:H7 at 4 °C and 10 °C

The GILs of *E. coli* O157:H7 inoculated on lettuce stored at 4 °C and 10 °C are shown in Figure 1. The figure presented clearly illustrates the relationship between inhibition levels and concentration dependence. The concentration of 420 ppm EP at 4 °C exhibited a greater inhibition rate compared to that observed at 10 °C at the end of the storage. On the fifth day, it was observed that the inhibition rate was higher for concentrations of 42 ppm and 105 ppm at 10 °C as compared to 4 °C. However, a concentration of 420 ppm exhibited a lower effect at 10 °C. The GILs obtained by the 42 ppm and 105 ppm EP concentrations at 10 °C had nearly the same rate.

Lettuce is a highly perishable vegetable, and as such, it is advisable to consume it in its fresh state. However, it can also be preserved for a few days by storing it at a temperature range of 4-5 °C (Hoza et al., 2020). The reason behind utilizing 10 °C in our research was to increase the impact of ethyl pyruvate on the duration of storage under elevated thermal conditions. Nevertheless, the data collected from the experiment revealed that the antimicrobial effectiveness of EP was relatively diminished at 10 °C. These findings align with the conclusions drawn by Ijabadeniyi et al. (2020). Besides storage temperature, the duration of storage and the concentration of antimicrobial agents are also influential factors that impact the efficacy of decontamination. The findings of this study provide clear evidence that higher concentrations of EP were effective in eliminating microbial activity. Furthermore, the extent of the logarithmic decrease in microbial activity was positively associated with the length of time the samples were stored.

Color and sensorial evaluation of lettuce

The color values (*L**, *a**, and *b**) of lettuce without *E. coli* O157:H7 after EP treatment are summarized in Table 2. A reduction in *L** values was observed on day 3 at 4 °C when comparing the control sample to those treated with 105 ppm and 420 ppm ($p<0.05$). The *L** value of lettuce samples was maintained by the 42 ppm and 105 ppm EP applications on the seventh day. A reduction in the *L** value was observed in both the control and 420 ppm EP-treated samples upon completion of the storage duration. The treatment of 420 ppm was found to exhibit the minimum *L** value. Regarding the storage at 10 °C, it was observed that the application of 105 ppm and 420 ppm EP resulted in the preservation of the *L** value on day 1, in contrast to the control group. However, a decline in the *L** value was noted in lettuce samples treated with 42 ppm EP. As a result of the presence of 420 ppm EP, a decrease in *L** value was seen at the end of storage. The EP with concentrations of 105 ppm and 420 ppm, in general, maintained the *L** value of lettuce during the storage period.

Applications of 105 ppm and 420 ppm EP increased *a** value during storage at 4 °C. The *a** value was better retained in the 42 ppm EP lettuce samples and the control sample. The observed increase in the *a**

Table 1. Inactivation of *E. coli* O157:H7 on lettuce by vaporized EP at 4 °C for 7 days and 10 °C for 5 days.

EP concentration (ppm)	*E. coli* O157:H7 count (log CFU/g)								
	4 °C					10 °C			
	0 day	1 day	3 days	5 days	7 days	0 day	1 day	3 days	5 days
0 (control)	10.4 ± 0.0^{aA}	10.1 ± 0.2^{aA}	10.5 ± 0.1^{aA}	10.4 ± 0.5^{aA}	10.3 ± 0.3^{aA}	10.4 ± 0.0^{bA}	10.4 ± 0.1^{bA}	10.8 ± 0.1^{aA}	10.7 ± 0.8^{aA}
42	10.4 ± 0.0^{aA}	10.2 ± 0.1^{abA}	9.7 ± 0.1^{bcB}	9.6 ± 0.8^{bcA}	9.5 ± 0.2^{cAB}	10.4 ± 0.0^{aA}	10.1 ± 0.2^{abAB}	9.9 ± 0.2^{bB}	9.4 ± 0.2^{cB}
105	10.4 ± 0.0^{aA}	9.9 ± 0.2^{abA}	9.4 ± 0.2^{abcB}	9.0 ± 0.6^{bcA}	8.8 ± 1.2^{cB}	10.4 ± 0.0^{aA}	10.1 ± 0.7^{aAB}	9.8 ± 0.5^{aB}	8.6 ± 0.1^{bB}
420	10.4 ± 0.0^{aA}	8.9 ± 0.7^{bB}	8.5 ± 0.4^{bC}	7.1 ± 1.6^{cB}	6.9 ± 0.4^{cC}	10.4 ± 0.0^{aA}	9.6 ± 0.1^{abB}	9.1 ± 0.1^{bcC}	8.5 ± 1.3^{cB}

$^{A-C}$: The same superscript uppercase letters show no significant ($p > 0.05$) differences between ethyl pyruvate concentrations within the same storage times. $^{a-c}$: The same superscript lowercase letters show no significant ($p > 0.05$) differences between storage times within the same ethyl pyruvate concentration.

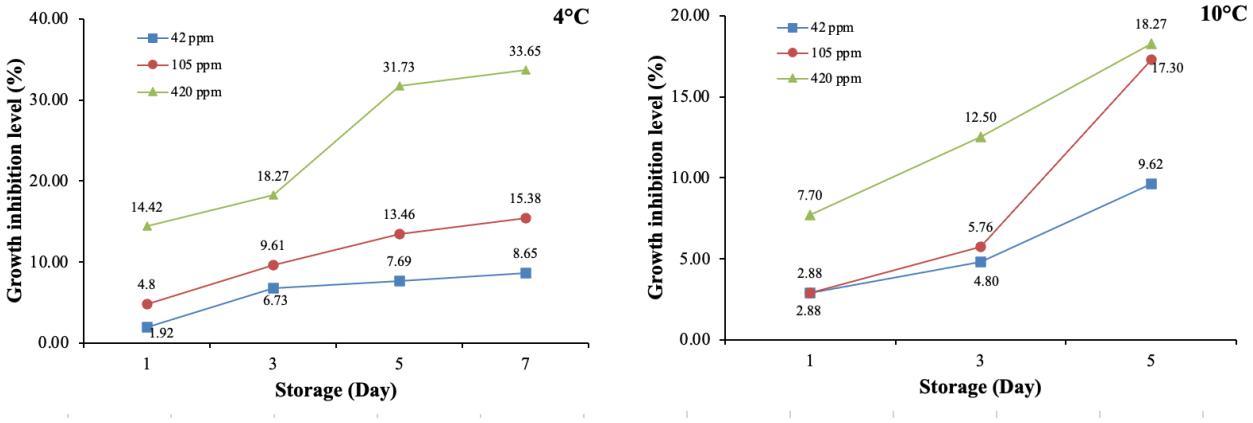

Figure 1. GILs of vaporized EP at different concentrations against *E. coli* O157:H7 on lettuce at 4 °C and 10 °C.

value corresponded with the rise in EP concentration, indicating an inability to sustain the green color. The 42 ppm EP application caused a decrease in *a** value during storage at 10 °C. Nevertheless, the observed rise was considered statistically insignificant on the 1st and 3rd days of storage ($p>0.05$). Upon completion of the storage period, it was observed that the utilization of 105 and 420 ppm EP increased the *a** value of samples ($p<0.05$).

The storage period exhibited an increase in the *b** value of samples due to the presence of 42 ppm EP at 4 °C. The samples exhibited comparable *b** values on the 3rd and 5th days of storage in the 105 ppm EP application. The 420 ppm EP showed the highest *b** value on the 7th day of storage. Upon completion of the storage period, it was noted that the utilization of 105 ppm of EP led to a reduction in the *b** value in comparison to the control sample. Regarding the storage conditions at 10 °C, no discernible variations were observed in the *b** values when comparing the control sample with the lettuce samples treated with EP, upon completion of the storage period ($p>0.05$).

The sensory scores of the samples are presented in Table 3. The data indicated a negative correlation between EP concentration and color scores relative to the control group at 4 °C. Upon evaluation of the concentrations concerning time, a decrease in the color scores was observed. After conducting an assessment of each concentration with respect to its corresponding time frame, no statistically significant change in the scores was detected ($p>0.05$). However, the application of 420 ppm EP on the 1st day and 105 ppm and 420 ppm EP applications on the 3rd day yielded a reduction in

Table 2. Color values for control and EP-treated lettuce stored at 4 °C and 10 °C.

	4 °C				10 °C			
Days	0 ppm (control)	42 ppm	105 ppm	420 ppm	0 ppm (control)	42 ppm	105 ppm	420 ppm
*L**								
0	57.11±5.1^{aB}				57.11±5.1^{aB}			
1	65.85±3.4^{aA}	61.33±3.2^{bAB}	65.89±1.3^{aA}	60.57±3.2^{bA}	58.34±2.3^{aAB}	53.95±1.0^{bB}	56.97±2.4^{aA}	58.29±2.3^{aA}
3	64.33±7.1^{aA}	60.21±5.9^{abAB}	55.94±4.4^{bcB}	51.55±5.4^{cC}	61.33±4.3^{aA}	57.33±1.6^{abA}	59.68±8.6^{aA}	53.37±7.4^{bA}
7	58.93±1.8^{bB}	64.14±7.1^{aA}	59.07±5.1^{aB}	53.35±4.0^{cBC}				
*a**								
0	-14.39±1.6^{aA}				-14.39±1.6^{aA}			
1	-16.12±0.6^{cB}	-12.45±2.7^{aA}	-15.53±0.4^{bcC}	-14.30±0.1^{bB}	-17.24±2.1^{aB}	-16.34±0.6^{aB}	-16.82±0.1^{aC}	-16.32±0.5^{aC}
3	-16.07±1.4^{cB}	-15.10±2.1^{bcB}	-12.59±3.6^{abAB}	-10.25±3.1^{aA}	-16.71±1.5^{bB}	-15.93±1.5^{bB}	-12.01±2.0^{aA}	-10.31±2.0^{aA}
7	-16.37±1.3^{bB}	-15.24±3.1^{bB}	-11.02±3.8^{aA}	-9.83±1.7^{aA}				
*b**								
0	25.28±3.5^{aB}				25.28±3.5^{aB}			
1	31.33±2.1^{aA}	23.16±4.1^{cC}	31.86±1.1^{aA}	27.50±0.7^{bAB}	32.44±3.9^{aA}	27.88±1.0^{bAB}	27.64±0.8^{bAB}	29.37±1.2^{bA}
3	29.53±4.7^{aA}	27.81±4.4^{abAB}	23.76±3.5^{bB}	24.45±4.6^{bB}	30.97±4.5^{aA}	28.60±3.5^{aA}	29.88±3.9^{aA}	26.71±5.8^{aAB}
7	29.47±3.4^{aA}	31.20±6.4^{aA}	23.72±6.2^{bB}	29.11±3.6^{aA}				

$^{A-C}$: The same superscript uppercase letters show no significant ($p > 0.05$) differences between ethyl pyruvate concentrations within the same storage times. $^{a-c}$: The same superscript lowercase letters show no significant ($p > 0.05$) differences between storage times within the same ethyl pyruvate concentration.

Table 3. Sensory scores of control and EP-treated lettuce stored at 4 °C and 10 °C.

	4 °C				10 °C			
Days	0 ppm (control)	42 ppm	105 ppm	420 ppm	0 ppm (control)	42 ppm	105 ppm	420 ppm
Color								
0	7.5±1.5^{aAB}				7.5±1.5^{aA}			
1	5.7±1.3^{bC}	7.3±0.9^{aA}	6.4±1.0^{bAB}	4.7±1.4^{cB}	6.2±1.3^{aB}	6.1±1.4^{aB}	4.9±1.2^{bB}	3.8±1.5^{cB}
3	8.2±1.2^{aA}	6.7±1.7^{aAB}	4.0±1.9^{bC}	3.2±1.9^{bC}	7.9±1.1^{aA}	7.1±1.1^{aAB}	4.9±2.1^{bB}	3.9±1.9^{bB}
7	6.7±1.5^{aBC}	5.6±2.0^{aB}	5.3±2.1^{abBC}	3.6±2.0^{bBC}				
Odor								
0	6.6±1.6^{aAB}				6.6±1.6^{aA}			
1	6.2±1.1^{aB}	6.5±1.1^{aA}	5.4±1.1^{aB}	3.6±2.1^{bB}	5.8±1.2^{aA}	5.5±1.3^{aB}	4.8±1.2^{abB}	3.7±1.8^{bB}
3	7.7±1.4^{aB}	6.7±1.2^{abA}	5.2±1.7^{bcB}	4.1±2.2^{cB}	7.0±1.4^{aA}	6.6±1.4^{aAB}	5.4±1.9^{abAB}	4.0±2.4^{bB}
7	6.2±1.6^{aA}	6.1±1.6^{aA}	4.4±2.0^{abB}	3.6±2.7^{bB}				
Appearance								
0	7.1±1.5^{aB}				7.1±1.5^{aA}			
1	5.4±1.3^{bcC}	7.0±1.0^{aA}	6.1±1.3^{abA}	4.7±1.6^{cB}	6.4±1.3^{aA}	6.0±1.3^{aA}	4.7±1.2^{bB}	3.8±1.1^{bB}
3	8.3±0.9^{aA}	6.1±2.3^{bAB}	3.9±1.9^{cB}	3.2±1.7^{cC}	7.1±1.9^{aA}	6.3±2.2^{abA}	5.0±1.9^{bcB}	3.8±1.9^{cB}
7	6.1±1.3^{aBC}	5.3±2.0^{abB}	4.1±2.0^{bcB}	3.5±1.9^{cBC}				
Texture								
0	7.3±1.2^{aA}				7.3±1.2^{aA}			
1	5.3±1.4^{bcB}	7.2±1.0^{aAB}	6.1±1.3^{bB}	4.7±1.5^{cB}	6.2±1.5^{aB}	6.3±1.4^{aA}	4.8±1.3^{bB}	4.3±1.6^{bB}
3	7.9±1.5^{aA}	6.0±1.7^{bB}	4.2±1.9^{cC}	3.1±1.8^{cC}	7.2±1.1^{aAB}	6.6±1.4^{aA}	4.9±2.3^{bB}	3.1±1.6^{cB}
7	5.9±1.7^{aB}	6.2±2.0^{aAB}	5.2±2.1^{abBC}	3.6±2.2^{bBC}				
Overall quality								
0	7.6±0.9^{aA}				7.6±0.9^{aA}			
1	5.4±1.3^{bcB}	7.1±1.0^{aAB}	6.2±1.0^{abB}	4.7±1.4^{cB}	6.2±1.3^{aB}	6.2±1.3^{aB}	5.0±1.2^{bB}	3.9±1.2^{cB}
3	8.1±1.1^{aA}	6.3±1.8^{bB}	4.6±2.1^{cC}	3.7±2.1^{cB}	7.3±1.2^{aA}	6.7±1.4^{aAB}	5.0±2.0^{bB}	3.4±1.8^{bB}
7	6.1±1.3^{aB}	6.2±1.7^{aB}	5.1±2.0^{abBC}	3.6±2.2^{bB}				

$^{A-C}$: The same superscript uppercase letters show no significant ($p > 0.05$) differences between ethyl pyruvate concentrations within the same storage times. $^{a-c}$: The same superscript lowercase letters show no significant ($p > 0.05$) differences between storage times within the same ethyl pyruvate concentration.

odor scores when compared to control samples. As for appearance and texture scores, the 42 ppm EP application demonstrated results that were identical to those of the control sample. The application of concentrations of 105 ppm and 420 ppm of EP led to a decrease in both visual appearance and textural quality ratings over the course of the storage duration. Looking at the overall acceptability scores, lower scores were obtained for all EP concentrations compared to the control sample. In addition, the control sample experienced a decrease in scores as a result of storage.

The lettuce samples exhibited better preservation of their color and odor properties when subjected to the 42 ppm EP application at 10 °C. The 420 ppm EP resulted in the lowest scores for both color and odor. The control sample and the samples treated with the 42 ppm EP exhibited greater appearance and structure scores during the storage period at 10 °C. After the storage period, a decline in both visual and structural characteristics was observed in comparison to the control group, as evidenced by the corresponding scores. At the end of the storage at 10 °C, a deterioration in both appearance and texture characteristics was observed in comparison to the control group, as evidenced by the corresponding scores. In the overall assessment, the lettuce samples that were treated with 105 ppm and 420 ppm EP were rated less acceptable than the samples from the control group.

The importance of preserving or improving the sensory attributes of fresh-cut fruits and vegetables during antimicrobial treatments is paramount due to the common consumption of these products in their raw state. The objective of the current study was to evaluate and compare the sensory and color traits of lettuce samples treated with EP and those that were untreated (control). The application of EP demonstrated a protective effect on the sensory and color properties of lettuce samples. The optimal attainment of color and sensory attributes is achieved through the utilization of 42 ppm EP. Previous research has indicated that baby spinach samples treated with EP exhibited lower sensory attributes compared to control samples when stored at 4 °C and 10 °C (Durak et al., 2012), which aligns with the findings of our own study.

CONCLUSION

This study investigated the antimicrobial activity of EP treatments at various concentrations against *E. coli* O157:H7 that were inoculated onto fresh lettuce stored for a duration of 7 days at 4 °C and for 5 days at 10 °C. The efficacy of EP at varying concentrations was observed in the inactivation of *E. coli* O157:H7 on fresh lettuce. The control sample exhibited a consistent bacterial load at 4 °C, whereas an increase in bacterial load was observed at 10 °C. While it is possible to observe bacterial growth at a storage temperature of 10 °C, it is clear that the utilization of EP demonstrates effective control over bacterial development in the samples. It was also determined that different storage temperatures also changed the effectiveness of EP. Although storing lettuce samples at 10 °C did not appear to be efficient in inactivating bacteria, 42 ppm and 105 ppm EP treatments at the end of storage showed a better rate of bacterial decrease than at 4 °C. Nevertheless, the results indicated that the 420 ppm EP yielded better results at 4 °C. Despite the successful inactivation of bacteria, increasing the concentration of EP had adverse effects on the color and sensory attributes of lettuce after the third day of storage.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the author.

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