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Antibacterial efficacy of ethyl pyruvate treatment against *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on cherry tomatoes

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ABSTRACT

Cherry tomatoes inoculated with *Escherichia coli* O157:H7 and *Salmonella* Typhimurium were treated with vaporised ethyl pyruvate (EP). The changes of microbial and organoleptic properties of the samples during storage were investigated at two temperatures (4 and 10 °C) and four EP concentrations (0, 42, 105, and 420 ppm) for 7 days at 4 °C and for 5 days 10 °C. After 3 days, 4.3 log and 3.6 log reductions in *E. coli* O157:H7 numbers were detected in cherry tomatoes treated with 42 ppm EP at 4 °C and at 10 °C, respectively. The highest EP treatment (420 ppm) led to 5.7 log CFU g^{-1} *E. coli* O157:H7 reduction after 1 day at 4 and 10 °C. The reduction of *Salmonella* Typhimurium on samples treated with 420 ppm EP was 7.7 log CFU g^{-1} after 1 day at 4 °C, and 6.9 log after 1 day at 10 °C. The treatment of EP can be effective at decreasing pathogen populations and can protect the organoleptic and colour properties of fresh produce.

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KEYWORDS

ethyl pyruvate, cherry tomatoes, decontamination, *E. coli* O157:H7, *Salmonella* Typhimurium

1. INTRODUCTION

Tomatoes are one of the most consumed fresh products whether fresh or processed (Valdivia-Nájar et al., 2017). Fresh products such as tomatoes, which are considered to be important nutrient sources and promote a healthy lifestyle are frequently consumed by the modern community around the world (Butscher et al., 2016). The health-promoting efficacy of fresh vegetables and fruit, which contain essential nutritional components such as vitamins, minerals, fibre, and polyphenols (Vojkovska et al., 2017; Akman et al., 2019), has been reported in many epidemiological studies (Castillejo et al., 2017). It is known that consumption of non-animal food is an effective way to prevent some diseases such as cancer, obesity, and cardiovascular diseases (Nou and Luo, 2010; Vojkovska et al., 2017). However, the number of foodborne disease outbreaks from contaminated fruit and vegetables has increased with the consumption of these products (Doyle and Erickson, 2008). Fresh vegetables and fruit can be contaminated with pre-harvest sources including soil, dust, faces, reconstituted fungicides and insecticides, contaminated irrigation water, raw or insufficiently composted manure, wild or domestic animals, and human handling (Tan et al., 2017). Since non-animal products are a vehicle for foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella* (Olaimat and Holley, 2012), it is recommended to use sterilising agents to decrease the activity of pathogenic microorganisms before consumption. Different sanitising methods, namely hydrogen peroxide treatment (Mei et al., 2017), sonication (Tan et al., 2017), treatment with ascorbic acid solution and pulsed lights (Hasan et al., 2017), plasma treatment (Butscher et al., 2016; Smet et al., 2017), chlorine washing (Gao et al., 2017), treatment of hydrosol (Sagdic, 2003), and several other treatment methods are used to reduce the microbial load in fresh products due to increasing public health concern related to the microbial safety of fruit and vegetables (Allende et al., 2008).

Ethyl pyruvate (EP), a simple derivative of pyruvic acid, is widely used as a therapeutic agent (Lee et al., 2017; Cetin et al., 2019) with multi-functional properties including anti-inflammatory, anti-oxidative, anti-apoptotic, and ion-chelating effects (Lee et al., 2017). Currently classified as generally safe by the US Food and Drug Administration, EP is used as a food additive in foods. In the present study, we focused on the efficiency of vaporised EP for decontamination of cherry tomatoes inoculated with two foodborne pathogens, *Salmonella* Typhimurium and *E. coli* O157:H7.

2. MATERIALS AND METHODS

2.1. Bacterial strains and culture preparation

Salmonella enterica subsp. *enterica* serovar Typhimurium ATCC 14028 and *E. coli* O157:H7 ATCC 25150 were obtained from Acibadem University (Istanbul, Turkey). The strains were cultured in Nutrient broth (Merck, Darmstadt, Germany) at 37 °C for 24 h. The initial inoculum

level of bacteria for spot inoculation method to contaminate cherry tomatoes was ~6 log CFU mL^{-1} for *E. coli* O157:H7 and ~8 log CFU mL^{-1} for *Salmonella* Typhimurium.

2.2. Sample preparation and inoculation procedure

Cherry tomatoes were purchased from a local grocery store (Istanbul, Turkey) on the day before the experiment and stored at 4 °C prior use. At the beginning, absence of *Salmonella* spp. and *E. coli* O157:H7 in the samples were confirmed. Cherry tomatoes were washed with tap water to clear undesired residues and minimise native microbial load for 10 min. They were then rinsed with deionised water and dried in a biosafety cabinet together with UV treatment for 30 min at room temperature. Cleaned and dried samples were spot-inoculated with 20 µL of bacteria with a micropipette on 10 to 15 points on the surface. Inoculated cherry tomatoes were dried in the biosafety cabinet for 2 h at ambient temperature.

2.3. Implementation of ethyl pyruvate

The vaporised ethyl pyruvate (EP, 98% purity; Sigma-Aldrich, St. Louis, USA) was administered to cherry tomatoes already inoculated with *E. coli* O157:H7 or *Salmonella* Typhimurium. Three whole inoculated cherry tomatoes were placed in a sterilised 2.6-L closed-lid food container (18.00 cm × 25.50 cm × 9.00 cm, Bora Plastic, Istanbul, Turkey). A sponge wetted with 20 mL of deionised water was put into each storage container to provide high humidity. In the amount determined by Durak et al. (2012), EP was absorbed by rough filter paper in each food container. After closing the container to prevent the release of the EP, EP-treated and control samples with microorganism were stored at 4 °C for 7 days and at 10 °C for 5 days.

2.4. Microbial analysis

Control and treated samples were homogenised for 2 min in sterile 0.1% peptone water (1:2, wt/vol) using a Stomacher apparatus (MiniMix 100, Interscience, St Nom, and France). Homogenised samples were serially diluted and appropriate dilutions were inoculated onto Xylose lysine deoxycholate agar (XLD agar, Merck, Darmstadt, Germany) and Sorbitol MacConkey agar (Merck, Darmstadt, Germany) for enumeration of *Salmonella* Typhimurium and *E. coli* O157:H7, respectively. The inoculated plates were incubated for 24 h at 37 °C and the presumptive colonies were counted as log CFU g^{-1} following the incubation.

2.5. Colour analysis

The colour of non-inoculated samples was quantified by colorimeter (Konica Minolta CR-400, Osaka, Japan). The values of L^*, a^*, and b^* (luminosity, chromaticity on a green (−) to red (+) axis, and chromaticity on a blue (−) to yellow (+) axis, respectively) were recorded in triplicates. The average L^*, a^*, and b^* values were calculated.

2.6. Sensory evaluation

The sensory analysis was performed on non-inoculated cherry tomatoes samples. Samples stored at 4 °C were tested on days 0, 1, 3, and 7, while samples stored at 10 °C were tested on days 0, 1, and 3. Samples were served in sterile food containers marked with three-digit numbers. Control (no EP treatment) and EP-treated samples (42, 105, and 420 ppm) were

simultaneously scored in terms of colour, odour, texture, and overall quality by an expert panel of 20 judges.

2.7. Statistical analysis

The statistical analysis was accomplished using the JMP statistical software (version 9.0, 2010, SAS Institute, Cary, NC) after all experiments were carried out three times on dependent samples. Differences between EP-treated and control samples regarding bacterial populations, colour and sensorial properties were determined using two-way analysis of variance and Tukey's multiple comparison test. The significance levels were set at 95% ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Inactivation of *E. coli* O157:H7 and Salmonella Typhimurium on cherry tomatoes

The effect of EP treatment on the count of *E. coli* O157:H7 for 7 days of storage at 4 °C and for 5 days of storage at 10 °C is presented in Table 1. The initial inoculation level of *E. coli* O157:H7 attached to the tomatoes was ~5.7 log CFU g^{-1}. The highest concentration of EP inhibited almost entirely the *E. coli* O157:H7 population on cherry tomatoe surfaces after one day of storage at 4 °C. Nevertheless, 42 ppm and 105 ppm concentrations of EP reduced *E. coli* O157:H7 population below the detection limit at 5th and 3rd day of storage at 4 °C, respectively. As the EP concentration increased, the inactivation time of *E. coli* O157:H7 decreased in cherry tomatoes stored at 10 °C, as well. After 1st, 3rd, and 5th day of storage, almost the entire population of *E. coli* O157:H7 on samples was inactivated with 420 ppm, 105 ppm, and 42 ppm EP, respectively ($P < 0.05$).

The survival incidence of *Salmonella* Typhimurium on cherry tomatoes after EP treatment during storage at 4 °C and 10 °C is presented in Table 2. Samples were inoculated with ~7.7 log CFU g^{-1} *Salmonella* Typhimurium. Treatment of 42 ppm EP led to a gradual decrease in *Salmonella* Typhimurium population during storage at 4 °C ($P < 0.05$). The 105 ppm and 420 ppm concentrations of EP reduced *Salmonella* Typhimurium population by 7.7 log CFU g^{-1} after the first day of storage ($P < 0.05$). The count of *Salmonella* Typhimurium population on the samples with 42, 105, and 420 ppm EP treatment at 10 °C was under the detection limit on day 5th, 5th, and 3rd, respectively. The antimicrobial activity of EP was found to be lower on the samples stored at 4 °C as compared to stored ones at 10 °C except for 105 ppm EP.

The 105 and 420 ppm EP treatments had greater effect on the inactivation of *Salmonella* Typhimurium than *E. coli* O157:H7 at 4 °C and 10 °C. In general, all EP concentrations had inhibitory effect on *E. coli* O157:H7 and *Salmonella* Typhimurium. However, the most effective concentration for *E. coli* O157:H7 was 420 ppm at both 4 °C and 10 °C. As for *Salmonella* Typhimurium, the best inactivation concentrations were 105 and 420 ppm at 4 °C, while 420 ppm at 10 °C.

There are different studies on the effect of EP on the inactivation of pathogenic bacteria in fresh produce (Durak et al., 2012; Tornuk and Durak, 2015; Bozkurt et al., 2016). Durak et al. (2012) carried out a study to observe the antimicrobial efficiency of EP on *E. coli* O157:H7 on green onion and spinach. A >4.7 log reduction on *E. coli* O157:H7 population on green onions after the end of the storage was succeeded. These results are in accordance with our results. The

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

EP concentration (ppm)	*E.coli* O157:H7 count (log CFU g^{-1})								
	4 °C					10 °C			
	Storage (day)					Storage (day)			
	0	1	3	5	7	0	1	3	5
0 (control)	5.7 ± 0.3^{aA}	5.7 ± 0.8^{aA}	4.7 ± 0.2^{bA}	5.2 ± 0.6^{abA}	5.1 ± 0.8^{abA}	5.7 ± 0.3^{aA}	5.8 ± 0.6^{aA}	6.0 ± 0.8^{aA}	4.8 ± 0.8^{bA}
42	5.7 ± 0.3^{aA}	4.5 ± 0.4^{bB}	1.4 ± 2.1^{cB}	$<0.5^{dB}$	$<0.5^{dB}$	5.7 ± 0.3^{aA}	3.7 ± 0.8^{bB}	$2.1 \pm 3.3b^{bB}$	$<0.5^{cB}$
105	5.7 ± 0.3^{aA}	4.5 ± 0.8^{bB}	$<0.5^{cC}$	$<0.5^{cB}$	$<0.5^{cB}$	5.7 ± 0.3^{aA}	0.6 ± 1.4^{bC}	$<0.5^{bC}$	$<0.5^{bB}$
420	5.7 ± 0.3^{aA}	$<0.5^{bC}$	$<0.5^{bC}$	$<0.5^{bB}$	$<0.5^{bB}$	5.7 ± 0.3^{aA}	$<0.5^{bC}$	$<0.5^{bC}$	$<0.5^{bB}$

$^{A-C}$: The same superscript uppercase letters in a column mean no significant ($P > 0.05$) differences. $^{a-c}$: The same superscript lowercase letters in a row mean no significant ($P > 0.05$) differences.

Table 2. Inactivation of *Salmonella* Typhimurium on cherry tomatoes by vaporised EP at 4 °C for 7 days and 10 °C for 5 days

EP concentration (ppm)	*Salmonella* Typhimurium count (log CFU g^{-1})								
	4 °C					10 °C			
	Storage (day)					Storage (day)			
	0	1	3	5	7	0	1	3	5
0 (control)	7.7 ± 0.5^{aA}	6.5 ± 0.6^{bA}	6.5 ± 0.3^{bA}	6.6 ± 0.2^{bA}	5.6 ± 0.1^{cA}	7.7 ± 0.5^{aA}	7.0 ± 0.4^{bA}	5.8 ± 0.2^{cA}	6.0 ± 0.1^{cA}
42	7.7 ± 0.5^{aA}	4.4 ± 0.1^{bB}	3.5 ± 0.3^{cB}	3.2 ± 0.2^{dB}	$<0.5^{eB}$	7.7 ± 0.5^{aA}	3.7 ± 0.4^{bB}	2.5 ± 0.3^{bcB}	$<0.5^{cB}$
105	7.7 ± 0.5^{aA}	$<0.5^{bC}$	$<0.5^{bC}$	$<0.5^{bC}$	$<0.5^{bB}$	7.7 ± 0.5^{aA}	3.4 ± 0.5^{bB}	0.5 ± 1.2^{bC}	$<0.5^{bB}$
420	7.7 ± 0.5^{aA}	$<0.5^{bC}$	$<0.5^{bC}$	$<0.5^{bC}$	$<0.5^{bB}$	7.7 ± 0.5^{aA}	0.8 ± 2.0^{bC}	$<0.5^{bC}$	$<0.5^{bB}$

$^{A-C}$: The same superscript uppercase letters in a column mean no significant ($P > 0.05$) differences. $^{a-c}$: The same superscript lowercase letters in a row mean no significant ($P > 0.05$) differences.

Table 3. Colour values for control and EP-treated tomatoes stored at 4 and 10 °C

Days	4 °C				10 °C			
	0 ppm (control)	42 ppm	105 ppm	420 ppm	0 ppm (control)	42 ppm	105 ppm	420 ppm
L^*								
0	38.19 ± 2.5^{aB}				38.19 ± 2.5^{aA}			
1	34.48 ± 1.0^{bC}	35.90 ± 0.8^{aC}	36.84 ± 1.2^{aC}	36.22 ± 1.2^{aA}	37.39 ± 1.6^{aA}	35.71 ± 0.3^{bC}	37.69 ± 0.8^{aB}	35.49 ± 2.0^{bB}
3	39.26 ± 2.0^{aAB}	38.44 ± 2.4^{aB}	39.12 ± 0.9^{aAB}	38.05 ± 1.1^{aA}	38.96 ± 1.5^{bA}	40.20 ± 0.5^{aA}	39.92 ± 0.4^{aA}	39.48 ± 0.9^{abA}
7	40.38 ± 1.1^{aA}	41.76 ± 0.6^{aA}	40.85 ± 1.4^{aA}	36.80 ± 4.3^{bA}				
a^*								
0	17.16 ± 4.4^{aC}				17.16 ± 4.4^{aA}			
1	23.33 ± 3.4^{abA}	23.57 ± 1.7^{abA}	25.39 ± 2.3^{aA}	21.38 ± 2.2^{bA}	18.63 ± 2.3^{bA}	19.98 ± 0.6^{bA}	23.32 ± 0.7^{aA}	23.00 ± 2.6^{aA}
3	21.33 ± 4.2^{aAB}	17.26 ± 3.8^{bB}	20.89 ± 2.9^{aB}	15.72 ± 2.6^{bB}	20.27 ± 3.9^{aA}	19.26 ± 1.4^{aAB}	19.77 ± 3.1^{aB}	16.37 ± 2.0^{bB}
7	19.29 ± 3.8^{aBC}	19.15 ± 2.6^{aB}	18.10 ± 2.0^{abBC}	15.61 ± 2.4^{bB}				
b^*								
0	9.39 ± 2.4^{aB}				9.39 ± 2.4^{aB}			
1	13.69 ± 2.8^{abA}	14.96 ± 0.5^{aB}	14.71 ± 1.3^{abA}	13.42 ± 0.5^{bA}	13.19 ± 0.9^{aA}	13.10 ± 1.4^{aA}	13.16 ± 1.6^{aA}	14.75 ± 2.6^{aA}
3	13.13 ± 1.9^{aA}	9.67 ± 2.5^{bB}	9.93 ± 1.0^{bB}	9.51 ± 0.9^{bB}	11.02 ± 2.9^{aB}	8.36 ± 1.1^{abB}	9.68 ± 4.4^{aB}	6.30 ± 1.9^{bC}
7	5.80 ± 1.7^{bC}	5.90 ± 1.0^{bC}	6.80 ± 1.5^{abC}	7.71 ± 2.4^{aC}				

$^{A-C}$: The same superscript uppercase letters in a column mean no significant ($P > 0.05$) differences. $^{a-c}$: The same superscript lowercase letters in a row mean no significant ($P > 0.05$) differences.

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

Days	4 °C				10 °C			
	0 ppm (control)	42 ppm	105 ppm	420 ppm	0 ppm (control)	42 ppm	105 ppm	420 ppm
Colour								
0	8.1 ± 0.9^{aA}				8.1 ± 0.9^{aA}			
1	6.2 ± 1.2^{bB}	7.1 ± 0.6^{abB}	7.2 ± 1.1^{aB}	6.4 ± 1.5^{bB}	7.3 ± 0.8^{aAB}	7.4 ± 0.8^{aAB}	7.2 ± 1.1^{aB}	7.7 ± 0.9^{aA}
3	7.6 ± 1.6^{aA}	7.3 ± 1.7^{aAB}	7.7 ± 1.1^{aAB}	6.9 ± 1.5^{aB}	6.9 ± 1.8^{aB}	7.0 ± 1.4^{aB}	7.7 ± 1.3^{aAB}	7.7 ± 1.1^{aA}
7	7.4 ± 1.2^{aA}	7.5 ± 0.8^{aAB}	6.8 ± 1.1^{abB}	6.2 ± 1.4^{bB}				
Odour								
0	7.1 ± 1.6^{aA}				7.1 ± 1.6^{aA}			
1	6.1 ± 1.0^{aA}	6.2 ± 1.0^{aA}	5.9 ± 1.4^{aB}	5.8 ± 1.3^{aB}	6.5 ± 1.1^{aA}	6.7 ± 1.0^{aA}	6.3 ± 1.4^{aA}	5.9 ± 1.8^{aA}
3	7.0 ± 1.6^{aA}	6.6 ± 1.5^{abA}	6.2 ± 1.3^{abAB}	5.3 ± 1.8^{bB}	6.9 ± 1.3^{aA}	6.4 ± 1.6^{aA}	5.9 ± 1.6^{aA}	6.0 ± 1.9^{aA}
7	6.9 ± 1.4^{aA}	6.3 ± 1.4^{aA}	6.1 ± 1.8^{aAB}	5.8 ± 2.3^{aAB}				
Appearance								
0	8.2 ± 1.0^{aA}				8.2 ± 1.0^{aA}			
1	6.5 ± 1.2^{aB}	6.7 ± 1.0^{aC}	7.3 ± 1.1^{aB}	7.2 ± 1.0^{aBC}	7.5 ± 1.0^{aB}	7.5 ± 0.8^{aB}	7.3 ± 0.9^{aB}	7.7 ± 0.6^{aA}
3	8.0 ± 1.2^{aA}	7.9 ± 1.3^{aAB}	7.7 ± 1.1^{aAB}	7.4 ± 1.1^{aAB}	7.6 ± 1.1^{aAB}	7.6 ± 1.0^{aAB}	7.8 ± 1.2^{aAB}	7.8 ± 0.8^{aA}
7	7.3 ± 1.5^{aAB}	7.2 ± 1.2^{aBC}	6.7 ± 1.3^{aB}	6.4 ± 1.2^{aC}				
Texture								
0	8.2 ± 1.3^{aA}				8.2 ± 1.3^{aA}			
1	6.5 ± 1.2^{aC}	7.0 ± 0.9^{aB}	6.9 ± 1.3^{aBC}	6.8 ± 1.1^{aBC}	7.2 ± 0.9^{aB}	7.3 ± 0.9^{aB}	7.0 ± 1.2^{aB}	7.7 ± 0.9^{aAB}
3	7.8 ± 1.2^{aAB}	7.8 ± 1.2^{aAB}	7.7 ± 1.1^{aAB}	7.3 ± 1.1^{aAB}	7.3 ± 1.1^{aAB}	7.6 ± 1.2^{aAB}	7.3 ± 1.3^{aAB}	6.9 ± 1.5^{aB}
7	7.0 ± 1.3^{aBC}	6.8 ± 1.3^{aB}	6.2 ± 1.5^{aC}	6.0 ± 1.8^{aC}				
Overall quality								
0	8.3 ± 0.8^{aA}				8.3 ± 0.8^{aA}			
1	6.5 ± 1.0^{aC}	6.8 ± 0.6^{aC}	6.7 ± 1.3^{aB}	6.7 ± 1.0^{aB}	7.3 ± 0.9^{aB}	7.2 ± 0.8^{aB}	6.9 ± 1.3^{aB}	7.4 ± 1.3^{aB}
3	8.0 ± 1.3^{aAB}	7.7 ± 1.2^{aAB}	7.2 ± 1.2^{aB}	6.9 ± 1.2^{aB}	7.4 ± 1.1^{aB}	7.4 ± 1.1^{aB}	7.2 ± 1.2^{aB}	6.8 ± 1.3^{aB}
7	7.4 ± 1.0^{aB}	6.9 ± 1.1^{abBC}	6.4 ± 0.8^{bB}	6.0 ± 1.2^{bB}				

$^{A-C}$: The same superscript uppercase letters in a column mean no significant ($P > 0.05$) differences. $^{a-c}$: The same superscript lowercase letters in a row mean no significant ($P > 0.05$) differences.

same results were also obtained from a study conducted by Tornuk and Durak (2015) who applied EP treatment on fresh parsley to inactivate *Staphylococcus aureus* and *E. coli* O157:H7.

3.2. Colour evaluation of cherry tomatoes

The colour values (L^*, a^*, and b^*) of cherry tomatoes without bacteria after EP treatment are summarised in Table 3. L^* values of samples treated with 420 ppm at 4 °C remained almost constant during the storage period but an increase was observed at 10 °C. a^* and b^* values of all EP treated samples decreased throughout storage at 4 °C and 10 °C. Since the natural colour of samples varied, no correlation could be found between a^* and b^* values of EP treated samples.

3.3. Sensory evaluation of cherry tomatoes

The sensorial scores of samples are given in Table 4. Upon evaluating the scores of colour considering storage time and EP concentration at 4 °C, the EP treatment, scores of cherry tomatoe samples given by the panellists decreased. However, there were no significant differences between control and EP treated samples at 10 °C ($P > 0.05$). Between the control and EP treated samples, regarding days of storage, no differences for odour, appearance, and texture were noticed at 4 and 10 °C. The overall acceptability of EP treated samples compared to control ones at both storage temperatures did not display a remarkable difference until the 3rd day.

4. CONCLUSIONS

Antibacterial efficiency of EP at different concentrations against *E. coli* O157:H7 and *Salmonella* Typhimurium and the effect of EP at different concentrations on colour and sensorial properties of cherry tomatoes were investigated at 4 and 10 °C. EP at different concentrations was highly effective in the inactivation of *E. coli* O157:H7 and *Salmonella* Typhimurium on cherry tomatoes. *Salmonella* Typhimurium was found to be more susceptible to EP treatment than *E. coli* O157:H7. The storage temperature had a significant effect on the antimicrobial activity of EP, which means a faster reduction in the number of microorganisms due to faster evaporation at 10 °C. In addition, EP treatment did not dramatically change the overall sensorial quality and colour properties.

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