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Effects of organic acid alone and in combination with H_2O_2 and NaCl on *Escherichia coli* O157:H7: An evaluation of antioxidant retention and overall acceptability in Basil leaves (*Ocimum basilicum*)

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ABSTRACT

In this study, the efficacy of household sanitizers application on reduction of *Escherichia coli* O157:H7, ascorbic acid, total phenolics, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and overall acceptability of inoculated fresh basil leaves (*Ocimum basilicum*), at temperature of 40 °C was investigated. Sanitizers containing lactic acid (LA), acetic acid (AA) and citric acid (CA) were used at concentration of 2%, individually or in combination with H_2O_2 (1% or 2%), and NaCl (7%). Control *a* and *b* were unwashed and washed leaves with distilled, deionized and sterilized water, respectively. All sanitizing treatments, in comparison to the control *a*, reduced the numbers of *E. coli* O157:H7 (0.24 ± 0.12–3.37 ± 0.48 log CFU/g) at day 1 (1 h after sanitizing). The lowest number of *E. coli* O157:H7 population (2.35 ± 0.26 log CFU/g) was observed by applying the LA + H_2O_2 (%2) treatment at first day of chilled storage. The highest amount of ascorbic acid (27.77 ± 0.06 mg/100 g), total phenolic (112.2 ± 0.5 mg gallic acid equivalents/100 g) and DPPH radical scavenging activity (95.2 ± 0.5%) was observed in control *a* at first day ($P < 0.05$). The results showed that the amount of ascorbic acid, total phenolics, DPPH radical scavenging activity and overall acceptability of basil leaves decreased during chilled storage. On day 2 of storage, the scores of sensory attributes for the control group were less than the minimum score of acceptance (i.e. 5 points). The results of this study indicated that LA + H_2O_2 (2%) treatment rendered the samples favorable in terms of overall appearance (≥5) up to 48 h.

1. Introduction

Vegetables are good source of vitamins, minerals, dietary fiber and phytochemicals which play important roles in maintaining human health (Dias, 2012). Basil (*Ocimum basilicum*) is widely cultivated worldwide. It can be used as both fresh vegetable and a medicinal herb (Zheljazkov et al., 2008). Basil is a good source of natural antioxidants, and contains significant amounts of important phytochemicals, such as phenolic compounds and ascorbic acid contents (Aburigal et al., 2017; Andrea and Jarmila, 2018). Fresh pre-packed basil can be potentially contaminated by enteric pathogens such as *Salmonella* spp. and pathogenic *Escherichia coli* (*E. coli*) (Delbeke et al., 2015). *Escherichia coli* O157:H7 (*E. coli* O157:H7) is the most common cause of hemorrhagic colitis and uremic hemicelluloses. On average, it causes 3890 cases of hemolytic uremic syndrome (HUS) globally and makes 230 deaths annually (Majowicz et al., 2014). Several notable factors can cause the cross-contamination of leafy greens with *Escherichia coli*. These factors include compost and soil fertilizers, the pollution of water resources and contact with infected people (Poimenidou et al., 2016).

Chlorinated water (in 50–200 ppm concentrate) is the most commonly used sanitizer in vegetables, and it can help to reduce the population of *E. coli* O157:H7 to < 1.6 log (Huang et al., 2012). However, various carcinogenic organochlorine compounds can be produced by the reaction of chlorine with organic compounds (CDC, 2009; Huang et al., 2012). Disinfecting vegetables with chlorinated water can have negative effects on the content of ascorbic acid and the external color of vegetables (Huang and Chen, 2011; Kenny and O'Beirne, 2009; Rahman et al., 2011). Therefore, it is necessary to introduce more effective sanitizers that are able to control microbial contaminations and can maintain nutritional and sensory properties of fresh vegetables. Numerous studies have indicated an exclusive focus on the antibacterial activity of alternative aqueous sanitizers including hydrogen peroxide, organic acid and NaCl (Hrenovic and Ivankovic, 2009; Huang and Chen, 2011; Lin et al., 2002; Nastou et al., 2012; Taormina and Beucha,

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1999; Van Haute et al., 2015).

Organic acids such as acetic, lactic and citric acid belong to the category of Generally Recognized as Safe (GRAS) substances. They have a long history in controlling *E. coli* O157: H7 populations (Lin et al., 2002). Furthermore, hydrogen peroxide has GRAS status and has been found to be effective on growth of *E. coli* O157: H7 (Raffellini et al., 2007; Huang et al., 2012; Jiang et al., 2017). The USDA-National Organic Program (Pushpinder et al., 2017) and European Food Safety Authority (EFSA, 2017) considered its possible use on leafy vegetables. Another compound that can counter *E. coli* O157: H7 is NaCl. Its mode of action is by affecting the osmotic properties of cells and by reducing the overall number of bacteria (Poimenidou et al., 2016).

Many reports have so far indicated that washing treatments affect antioxidant retention and sensorial properties of edible products, and the abilities of such treatments in inhibiting *E. coli* O157:H7 are diverse (Kenny and O'Beirne, 2009; Kim and Rhee, 2015; Poimenidou et al., 2016). Sanitizers and pathogens induce specific changes in cellular metabolism within vegetable tissues; other changes may occur to enzyme activities, especially phenylalanine ammonia lyase (PAL), and polyphenol oxidase (PPO) (Ngadze et al., 2012; Roura et al., 2008). PAL is the key enzyme in the synthesis of phenols and lignin (Galani et al., 2017). Whenever a tissue is infected or wounded, PPO level increase and cause the production of quinones by oxidizing the phenols, thereby resulting in browning (Ngadze et al., 2012). The effects of sanitizers on vegetables depend on the target microorganism, the initial number of microorganisms on the leafy sample, the method of inoculation, the type of treatment or sanitizer and the surface characteristics of the green leaf (Macarisin et al., 2013; Rico et al., 2007; Sanz et al., 2002). A relevant report suggested that mild heat (40–50 °C) can increase the antimicrobial effects of sanitizers and can improve the quality of leafy vegetables (Huang and Chen, 2011; Delaquis et al., 2002).

In this study, we examined different sanitizer containing H_2O_2, NaCl and various organic acids (as outlined in Table 1) on basil leaves. The effects of sanitizers were investigated on the inhibition of *E. coli* O157:H7 (IBRC-M: 10708) and protective effects on contents of vitamin C, phenolics, antioxidants and the acceptance of fresh basil leaves during 72 h of storage at 4 °C.

2. Materials and methods

2.1. Bacterial strain and activation procedure

The *E. coli* O157: H7 (IBRC-M: 10708), which is resistant to nalidixic acid, was purchased from a valid scientific center in lyophilized form. The bacteria was activated according to the procedure documented by Huang and Chen (2011), with slight modifications. The cells were transferred to 10 mL of tryptic soy broth (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract and with 50 μg/mL nalidixic acid (Fisher Scientific, Hampton, NH) (TSBYE-N). The mixture was then incubated at 37 °C for 24 h. To obtain single colonies, 100 μL of the 24-hour old culture was incubated in a tryptic soy agar supplemented with 0.6% yeast extract and 50 μg/mL nalidixic acid (TSAYE-N), and the plates were incubated at 37 °C for 24 h. Then, one colony was transferred to 9 mL of TSBYE-N medium and incubated at 37 °C for 24 h. To obtain the culture overnight, 1 mL of aliquot culture was transferred to 9 mL of TSBYE-N and was re-incubated under same conditions. In order to harvest the cells, 30 mL of the culture was centrifuged (Eppendorf 5810, Germany) at 3500*g* for 15 min, and the plate was washed twice by a physiological serum (0.9%).

To obtain the population growth curve, the serial dilutions were prepared from the pellets. The absorbance value of each aliquot was measured using the UV-VIS spectrophotometer device (Cecil model, England) at a wavelength of 620 nm. Then, 100 μL of each aliquot was poured into the TSAYE-N. Colonies were counted after the plates had been incubated at 37 °C for 24 h to determine the bacterial population. Then, the cell suspension (10^9 CFU/mL) was prepared according to a working curve.

Nomenclature used in text, tables and figures.

Treatment	Abbreviation	pH
No washing	Control *a*	–
Sterile deionized distilled water	Control *b*	6.9
Lactic acid (2% v/v)	LA	1.9
Acetic acid glacial (2% v/v)	AA	2.7
Citric acid (2% v/v)	CA	1.7

Table 1
Effect of different sanitizing treatments on the population of *E. coli* O157:H7 (CFU/g) in basil leaves, during storage time at (4 ± 1 °C).

Treatment	Time (day)			
	1	2	3	4
Control *a*	$5.73 \pm 0.18^{D,a}$	$6.47 \pm 0.15^{C,a}$	$7.07 \pm 0.23^{B,a}$	$7.64 \pm 0.11^{A,a}$
Control *b*	$5.43 \pm 0.12^{D,ab}$	$6.36 \pm 0.09^{C,a}$	$7.09 \pm 0.22^{B,a}$	$8.02 \pm 0.20^{A,a}$
LA	$4.26 \pm 0.10^{B,efg}$	$4.42 \pm 0.12^{AB,gh}$	$4.60 \pm 0.20^{AB,i}$	$4.69 \pm 0.269^{A,ghi}$
AA	$3.56 \pm 0.10^{C,hi}$	$4.16 \pm 0.11^{B,hi}$	$4.60 \pm 0.21^{AB,i}$	$4.90 \pm 0.18^{A,g}$
CA	$3.98 \pm 0.12^{C,g}$	$4.29 \pm 0.11^{BC,h}$	$4.58 \pm 0.20^{B,i}$	$4.79 \pm 0.28^{A,gh}$
Na	$4.87 \pm 0.14^{D,c}$	$5.45 \pm 0.13^{C,bcd}$	$5.95 \pm 0.22^{B,cd}$	$7.09 \pm 0.19^{A,b}$
H_2O_2 (1%)	$4.71 \pm 0.11^{D,cd}$	$5.39 \pm 0.14^{C,cd}$	$6.32 \pm 0.21^{B,bc}$	$6.95 \pm 0.24^{A,bc}$
H_2O_2 (2%)	$4.50 \pm 0.15^{D,de}$	$5.14 \pm 0.22^{C,de}$	$6.09 \pm 0.19^{B,cd}$	$6.78 \pm 0.25^{A,bc}$
LA + H_2O_2 (%1)	$4.27 \pm 0.09^{C,efg}$	$4.64 \pm 0.25^{C,fg}$	$5.09 \pm 0.20^{B,gh}$	$5.52 \pm 0.17^{A,f}$
AA + H_2O_2 (%1)	$3.69 \pm 0.11^{C,h}$	$5.10 \pm 0.13^{B,de}$	$5.3 \pm 0.22^{B,fg}$	$5.57 \pm 0.18^{A,f}$
CA + H_2O_2 (%1)	$4.30 \pm 0.17^{D,ef}$	$4.88 \pm 0.27^{C,ef}$	$5.25 \pm 0.19^{B,fg}$	$5.56 \pm 0.15^{A,f}$
LA + H_2O_2 (%2)	$2.35 \pm 0.26^{D,j}$	$3.20 \pm 0.30^{C,j}$	$3.75 \pm 0.20^{B,k}$	$4.28 \pm 0.42^{A,ij}$
AA + H_2O_2 (%2)	$4.67 \pm 0.31^{C,ef}$	$5.37 \pm 0.23^{B,cd}$	$5.55 \pm 0.22^{AB,ef}$	$5.68 \pm 0.18^{A,f}$
CA + H_2O_2 (%2)	$4.11 \pm 0.22^{D,efg}$	$5.58 \pm 0.24^{C,bc}$	$6.15 \pm 0.20^{B,bcd}$	$6.69 \pm 0.25^{A,bcd}$
LA + Na	$3.49 \pm 0.13^{B,hi}$	$3.87 \pm 0.13^{A,i}$	$3.94 \pm 0.21^{A,jk}$	$3.99 \pm 0.44^{A,j}$
AA + Na	$3.25 \pm 0.24^{C,i}$	$5.76 \pm 0.05^{B,b}$	$6.04 \pm 0.30^{AB,cd}$	$6.30 \pm 0.20^{A,de}$
CA + Na	$5.49 \pm 0.12^{B,ab}$	$6.29 \pm 0.26^{A,a}$	$6.50 \pm 0.19^{A,b}$	$6.66 \pm 0.25^{A,cd}$
LA + H_2O_2 (%1) + Na	$3.48 \pm 0.12^{C,hi}$	$3.90 \pm 0.15^{B,i}$	$4.15 \pm 0.21^{B,j}$	$5.10 \pm 0.19^{A,g}$
AA + H_2O_2 (%1) + Na	$3.53 \pm 0.14^{C,hi}$	$4.29 \pm 0.24^{B,h}$	$4.80 \pm 0.20^{A,hi}$	$5.09 \pm 0.27^{A,g}$
CA + H_2O_2 (%1) + Na	$3.66 \pm 0.28^{C,h}$	$4.48 \pm 0.22^{B,gh}$	$4.60 \pm 0.22^{B,i}$	$5.08 \pm 0.18^{A,g}$
LA + H_2O_2 (%2) + Na	$4.91 \pm 0.14^{C,e}$	$5.59 \pm 0.25^{B,bc}$	$5.85 \pm 0.20^{AB,de}$	$6.10 \pm 0.19^{A,e}$
AA + H_2O_2 (%2) + Na	$3.44 \pm 0.18^{B,hi}$	$3.94 \pm 0.22^{B,i}$	$3.64 \pm 0.24^{B,k}$	$4.19 \pm 0.16^{A,j}$
CA + H_2O_2 (%2) + Na	$5.23 \pm 0.25^{C,b}$	$5.66 \pm 0.18^{B,bc}$	$5.92 \pm 0.23^{AB,cd}$	$6.28 \pm 0.20^{A,de}$

The values are the means ± SD of triplicate experiments. Means followed by different uppercase and lowercase letters indicate significant differences in row and column, respectively ($P < 0.05$).

NaCl (7% w/v)	NaCl	6.9
H_2O_2 (1% v/v)	H_2O_2 (1%)	4.7
H_2O_2 (2% v/v)	H_2O_2 (2%)	4.5
Lactic acid (2% v/v) + H_2O_2 (1% v/v)	LA + H_2O_2 (%1)	2.1
Acetic acid glacial (2% v/v) + H_2O_2 (1% v/v)	AA + H_2O_2 (%1)	2.8
Citric acid (2% w/v) + H_2O_2 (1% v/v)	CA + H_2O_2 (%1)	1.8
Lactic acid (2% v/v) + H_2O_2 (2% v/v)	LA + H_2O_2 (%2)	2.2
Acetic acid glacial (2% v/v) + H_2O_2 (2% v/v)	AA + H_2O_2 (%2)	2.9
Citric acid (2% w/v) + H_2O_2 (2% v/v)	CA + H_2O_2 (%2)	1.9
Lactic acid (2% v/v) + NaCl (7% w/v)	LA + Na	1.6
Acetic acid glacial (2% v/v) + NaCl (7% w/v)	AA + Na	2.3
Citric acid (2% w/v) + NaCl (7% w/v)	CA + Na	1.5
Lactic acid (2% v/v) + H_2O_2 (1% v/v) + NaCl (7% w/v)	LA + H_2O_2 (%1) + NaCl	1.6
Acetic acid glacial (2% v/v) + H_2O_2 (1% v/v) + NaCl (7% w/v)	AA + H_2O_2 (%1) + NaCl	2.3
Citric acid (2% w/v) + H_2O_2 (1% v/v) + NaCl (7% w/v)	CA + H_2O_2 (%1) + NaCl	1.5
Lactic acid (2% v/v) + H_2O_2 (2% v/v) + NaCl (7% w/v)	LA + H_2O_2 (%2) + NaCl	1.6
Acetic acid glacial (2% v/v) + H_2O_2 (2% v/v) + NaCl (7% w/v)	AA + H_2O_2 (%2) + NaCl	2.3
Citric acid (2% w/v) + H_2O_2 (2% v/v) + NaCl (7% w/v)	CA + H_2O_2 (%2) + NaCl	1.5

2.2. Preparation of sanitizers

Sanitizers were prepared according to Table 1, whereby distilled, deionized and sterilized water were applied for 5 min before washing. Acetic acid (AA), lactic acid (LA), citric acid (CA), NaCl and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich (USA). The mentioned substances were incorporated into sterilized deionized water so as to formulate solutions of 2% (w/v) AA, 2% (v/v) LA, 2% (v/v), 2% (w/v) CA, 1 and 2% (v/v) H_2O_2 and 7% (w/v) NaCl. The sanitizers were used as mixtures of selected organic acids, along with NaCl and hydrogen peroxide, which were integrated by being dissolved in sterilized deionized water in order to reach the ultimate concentration (Table 1). The pH values of sanitizers were measured by a digital pH meter after calibration (Labnic, USA).

2.3. Preparation of basil leaves

Basil was sampled from a commercial greenhouse on the day of evaluation. The plants have not been treated with any herbicides or pesticides. The leaves were separated from the stems and the healthy specimens (which were not wilted, but were healthy and glossy). They were kept in 30 g sterilized packages (at 4 ± 1 °C, for a maximum duration of 1 h) until the beginning of the tests.

2.4. Inoculation of basil leaves

The leaves were inoculated by bacteria via the spraying method. For this purpose, 30 mL of the cell suspension (10^9 CFU/mL) was added to a sterilized physiological serum (0.9%). It was reached to volume of 3 L to obtain the final concentration of bacterial cells of about 10^6 CFU/mL. The cell suspension was sprayed on vegetables under a hood, so that both sides of the leaves were completely soaked under sterile conditions. The inoculated leaves were dried at ambient temperature on a sterile steel net for 30 min and were transferred to zipper plastic bags (sterilized with alcohol). Basil leaves were placed in a refrigerator at 4 ± 1 °C for 24 h in order to allow the bacteria to be fixed on the leaf surface (Lang et al., 2004).

2.5. Sanitizing

Inoculated basil leaves (4 ± 1 °C) were kept at ambient temperature for 30 min. Ten grams of the sample was transferred to each sterile glass container, and 400 mL of the fresh sanitizer was added at 40 °C (Table 1). After 30 s, the basil leaves were separated by sterile metal nets and immersed in sterile ice water for 30 s. Decanted basil leaves were packed in zipper bags (sterilized with alcohol). The control '*b*' sample was disinfected with sterile, deionized, distilled water. The control '*a*' sample was packed without exposing to any disinfection. A total of 276 bags were stored at 4 ± 1 °C for 72 h. Microbial and chemical analysis was performed at 1 (1 day), 24 (2 day), 48 (3 day) and 72 h (4 day) after cold storage on all of samples.

2.6. Inactivation of Escherichia coli O157: H7

The number of *E. coli* O157:H7 bacteria were counted according to the method used by Huang et al. (2012) with slight modifications. For this purpose, 5 g of basil leaves was transferred to a stomach bag, and then 95 cc of the physiological serum (0.9%) was added. Homogenization was carried out using a stomacher instrument (Interscience, France) for 4 min. Then, the number of cells was counted according to the procedure outlined in Section 2.1, and then converted to log CFU/g.

2.7. Preparation of extracts

The extracts were prepared according to the method used by Murugan et al. (2013) with slight modifications, and it was performed in order to measure the amounts of ascorbic acid, total phenolic content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging in the basil leaves. For this purpose, 0.5 g of basil leaves were cut and divided into small sizes. They were homogenized with 5 mL of methanol (96%) in a porcelain mortar. To separate the extract, a centrifuge machine (Eppendorf 5810, Germany) operated at 3500 g for 10 min. The process was repeated and the extract was transferred to an oven (40 °C) in order to remove the solvent.

2.8. Determination of ascorbic acid

Ascorbic acid was quantified according to the method used by Chang et al. (2006) with slight modifications. About 1 g of the basil leaves and 10 mL of metaphosphoric acid (1%) (w/v) were mixed and homogenized. The homogenate was centrifuged at 8000*g* for 20 min. 1 mL of the supernatant was mixed with 4 mL of 0.5 mM 2,6-dichlorophenolindophenol, and the absorbance of the mixture was measured by a spectrophotometer at 520 nm.

2.9. Determination of total phenolic content

The total phenolic contents of the basil leaf extracts were quantified by the Folin–Ciocalteu method according to the procedures used by Duarte-Almeida et al. (2006). Accordingly, 0.5 mL of methanolic extract was added to 0.1 mL of Folin–Ciocalteu with 6 mL of distilled water. Then, 2 mL of sodium carbonate (15%) and 10 mL of distilled water were added and then mixed at room temperature. Incubation was performed in the dark at 37 °C for 2 h, and the absorbance was measured at 750 nm. Gallic acid (Sigma-Aldrich, USA (was used as a standard and Total phenolic contents were expressed as milligrams of gallic acid equivalents per 100 g of fresh basil leaves (mg GAE/100 g).

2.10. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Antioxidant activity was assessed in terms of hydrogen donating or radical scavenging abilities of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method used by Chang et al. (2006). For this purpose, one milliliter of the methanolic extract solution was added to 2 mL of DPPH (which had a concentration of 0.1 mM in methanol). The absorbance was read at 517 nm, and then the percentage of DPPH· scavenging activity was measured according to the following formula:

$$\text{DPPH}^{\cdot}\text{scavenging activity (\%)} = [(\Delta A_{515nm}\text{ of control} - \Delta A_{515nm}\text{ of sample}) / \Delta A_{515nm}\text{ of control}] \times 100$$

2.11. Evaluation of overall acceptability

The overall acceptability of the product (i.e. brightness, aroma and attractiveness for consumer purchase) was tested according to the procedures outlined by Majumdar et al. (2010). To simulate the randomness of consumers, a panel of 20 individuals which comprised of students and staff members (male and female), aged between 21 and 35, were asked to evaluate the samples. The tests were performed using a 9-point hedonic scale, where 9 meant "like extremely" and 1 meant "dislike extremely". The minimum acceptable score was 5.

2.12. Statistical analysis

All of the above experiments were replicated three times. The results were analyzed by SAS software (version 9.1) using the factorial experiment in a completely randomized design (CRD). The *E. coli* O157: H7 population which had been obtained during the course of several days was converted to log 10 CFU/g. The comparisons of mean values of chemical and microbial characteristics were performed by Duncan's test ($P < 0.05$).

3. Results and discussion

3.1. Anti-pathogenic effect of sanitizer

The initial population of *E. coli* O157: H7 on inoculated basil leaves was 5.73 ± 0.31 log CFU/g before using sanitizers on the samples. The highest number of *E. coli* O157:H7 were observed in control *a* (not-treated) and control *b* (disinfected with sterile deionized distilled water) on all days of the evaluation and CA + Na samples (at 1 and 2 day) ($P < 0.05$) (Table 1). Nastou et al. (2012) reported disinfection of salad vegetables with water reduced *Listeria monocytogenes* populations by about 0.5 log CFU/g when dipped in water for 5 min and their results were similar to the same category of results in this study.

The results showed that the number of cells counted in the samples of the control *a*, *b*, NaCl, H_2O_2 (1% and 2%), CA + H_2O_2 (1%) and LA + H_2O_2 (2%) was significantly increased during the storage of samples ($P < 0.05$). In other samples, there was no statically significant difference in cell growth on some days of the evaluation period. This may be due to changes in cellular mechanisms as a result of environmental stress. It is known that stress conditions encourage cells to develop defense mechanisms through the coordinated expression of genes. These evolutionary protective or adaptive systems may help the cells to modify their environments and thus survive (Chang et al., 2006).

In organic acid group, treating the samples with acetic acid showed the highest inhibition effects on cell growth at 1 day, and there appeared no significant difference between citric acid and lactic acid sanitizers. Huang and Chen (2011) reported immediately after sanitizing baby spinach by organic acid (2%, 5 min, 40 °C), lactic acid > citric acid > acetic acid reduced the population of *E. coli* O157:H7, respectively. Differences between the results are probably due to the difference in the type of vegetables and the duration of disinfection (Nastou et al., 2012; Poimenidou et al., 2016). Organic acid is capable of destroying the cell membrane, thereby resulting in a more acidic climate within the inter-cellular environment and inhibiting essential metabolic reactions. It can cause the accumulation of toxic anions (Brul and Coote, 1999) and may lead to inhibition of cell growth. This feature is influenced by some factors such as undissociated acids, chain lengths, cell membrane permeability and environmental conditions (Doores, 2005). There was no significant difference between the effects of 1% and 2% H_2O_2 on cell growth at all times. This result is in agreement with those reported by Huang and Chen (2011). The application of 1% and 2% concentrations of H_2O_2 in combination with organic acids showed different capabilities of inhibiting *E. coli* O157: H7 growth. Binary combination treatments of LA + H_2O_2 (2%) showed synergistic effects on controlling the *E. coli* O157: H7 growth which led to greatest logarithmic reductions in the *E. coli* O157:H7 ≥ 3.27 log CFU/g when compared to control *a* on first and second days of the evaluation. Relevantly, Van Haute et al. (2015) stated that the decomposition of hydrogen peroxide produces OH radicals, but the interactions between compounds in the sanitizer can affect antibacterial potential.

A strong effect on the decline of the *E. coli* O157: H7 population was not observed (< 1.12 Log CFU/g) as a result of disinfecting the basil leaves with NaCl 7% (w/v). This can be due to the osmotolerant feature of *E. coli* O157: H7 bacteria (Hrenovic and Ivankovic, 2009). It is well known that NaCl can increase osmotic pressure which results in decreased water activity of the medium surrounding the microorganisms. However, systems of osmoregulation in microorganisms make an equilibrium of osmotic pressure by the active transfer of charged solutes such as K^+, glutamate, trehalose, proline, glycine, betaine and carnitine (Pichereau et al., 2000). Adding NaCl to lactic acid synergistically affected the antimicrobial activity, but a negative effect was observed when it was used in combination with citric and acetic acids.

Ternary combinations of sanitizers showed different effects on the cell growth reduction. It is may be due to different levels of destruction osmoregulatory systems by heat shock and pH stress, which can finally change membrane functionality and inhibition of cell growth (Delaney et al., 1993; Brown et al., 1997).

3.2. Ascorbic acid content

Ascorbic acid is one of the most important antioxidants in plant sources that can prevent the development of cancers, diabetes and cardiovascular diseases (Dewhirst et al., 2017). Changes in ascorbic acid values in basil leaves are shown in Table 2. In all samples, the amount of ascorbic acid significantly decreased during the storage time ($P < 0.05$). Kenny and O'Beirne (2009) reported similar results with regard to the washing of lettuce with chlorine water or tap water. Factors such as light, oxygen, ambient heat and long-term maintenance can adversely reduce the level of ascorbic acid in vegetables (Kapur et al., 2012). The highest amount of ascorbic acid was recorded in control a (27.77 ± 0.06 mg/100 g) on the first day ($P < 0.05$). Dewhirst et al. (2017) argued that the act of disinfection puts mechanical stress on vegetables and reduces the amount of vitamin C. Another reason might be the occurrence of severe leaching and brown enzymatic reactions (Lang et al., 2004). The lowest values of ascorbic acid were observed in samples when treated by H_2O_2 (1 and 2%) on the 4th day of storage time. Harper et al. (2007) argued that ascorbic acid is degraded in the presence of hydrogen peroxide at various concentrations.

In the group containing organic acid treatments, the strongest protective effect was achieved by LA treatment on the first day, and also by using CA on the 2th, 3th and 4th days ($P < 0.05$). Adding H_2O_2 (1%) to sanitizers containing acetic acid (AA + H_2O_2 (1%)) and at 2% to citric acid (CA + H_2O_2 (2%)) showed negative effect on the amount of ascorbic acid about 8.01–48.56% and 1.26–37.63% respectively. The comparison of ascorbic acid levels in other treatments containing organic acid + hydrogen peroxide (1 and 2%) showed different protective effects ($P < 0.05$). Harper et al. (2007) argued that the presence of hydrogen peroxide can rapidly degrade ascorbic acid, whereas hydrogen peroxide can be affected by organic acid through the interactions with antioxidant substances such as anthocyanin and flavonoids which, in turn, affect the oxidation of ascorbic acid.

The NaCl treatment showed a low protective effect on ascorbic acid. In this regard, Babalola et al. (2010) reported the disinfection of leafy vegetables with a solution containing (10% w/v) NaCl which reduced the amount of ascorbic acid. Singh et al. (2015) argued that changing

Table 2
Effect of different sanitizing treatments on ascorbic acid (mg/100 g fresh wt.) in basil leaves, during storage time at (4 ± 1 °C).

Treatment	Time (day)			
	1	2	3	4
Control *a*	27.77 ± 0.06^{A,a}	9.15 ± 0.08^{B,t}	6.41 ± 0.02^{C,r}	3.77 ± 0.07^{D,r}
Control *b*	19.50 ± 0.09^{A,m}	7.51 ± 0.05^{B,u}	5.47 ± 0.02^{C,s}	3.43 ± 0.08^{D,s}
LA	25.54 ± 0.04^{A,c}	13.93 ± 0.02^{B,n}	12.60 ± 0.02^{C,l}	11.25 ± 0.04^{C,i}
AA	19.60 ± 0.04^{A,l}	15.11 ± 0.01^{B,k}	13.12 ± 0.01$^{C,\ k}$	11.14 ± 0.01^{D,j}
CA	23.72 ± 0.02^{A,f}	17.01 ± 0.01^{B,f}	14.48 ± 0.01^{C,h}	11.96 ± 0.01^{D,h}
Na	16.80 ± 0.06^{A,s}	7.41 ± 0.07^{B,v}	5.12 ± 0.03^{C,t}	3.1 ± 0.05^{D,t}
H_2O_2 (1%)	15.11 ± 0.02^{A,u}	11.41 ± 0.06^{B,p}	3.81 ± 0.04^{C,u}	2.35 ± 0.08^{D,u}
H_2O_2 (2%)	15.33 ± 0.04^{A,t}	10.91 ± 0.05^{B,r}	3.23 ± 0.01^{C,v}	2.41 ± 0.13^{D,u}
LA + H_2O_2 (%1)	25.39 ± 0.07^{A,d}	16.62 ± 0.01^{B,h}	11.45 ± 0.05^{C,m}	6.55 ± 0.01^{D,p}
AA + H_2O_2 (%1)	18.02 ± 0.02^{A,p}	11.32 ± 0.01^{B,q}	8.53 ± 0.01^{C,q}	5.73 ± 0.01^{D,q}
CA + H_2O_2 (%1)	23.98 ± 0.02^{A,e}	18.08 ± 0.02^{B,d}	13.88 ± 0.04^{C,i}	9.75 ± 0.03^{D,l}
LA + H_2O_2 (%2)	19.15 ± 0.01^{A,n}	16.84 ± 0.02^{B,g}	14.71 ± 0.01^{C,e}	12.57 ± 0.03^{D,g}
AA + H_2O_2 (%2)	17.15 ± 0.02^{A,q}	15.65 ± 0.03^{B,i}	14.61 ± 0.01^{C,f}	13.56 ± 0.02^{D,d}
CA + H_2O_2 (%2)	23.43 ± 0.02^{A,h}	13.02 ± 0.02^{B,o}	10.25 ± 0.01^{C,o}	7.46 ± 0.01^{D,m}
LA + Na	26.55 ± 0.02^{A,b}	20.13 ± 0.01^{B,a}	18.32 ± 0.06^{C,a}	16.82 ± 0.01^{D,a}
AA + Na	17.10 ± 0.02^{A,r}	14.65 ± 0.03^{B,l}	10.75 ± 0.02^{C,n}	6.85 ± 0.05^{D,o}
CA + Na	18.22 ± 0.04^{A,o}	10.54 ± 0.03^{B,s}	8.83 ± 0.02^{C,p}	7.08 ± 0.08^{D,n}
LA + H_2O_2 (%1) + Na	19.73 ± 0.02^{A,k}	15.47 ± 0.01^{B,j}	13.72 ± 0.01^{C,j}	11.95 ± 0.02^{D,h}
AA + H_2O_2 (%1) + Na	21.10 ± 0.02^{A,i}	19.25 ± 0.01^{B,b}	17.64 ± 0.01^{C,c}	16.01 ± 0.02^{D,c}
CA + H_2O_2 (%1) + Na	23.97 ± 0.03^{A,e}	20.09 ± 0.01^{B,a}	18.03 ± 0.01^{C,b}	16.25 ± 0.06^{D,b}
LA + H_2O_2 (%2) + Na	21.10 ± 0.02^{A,i}	18.55 ± 0.03^{B,c}	15.68 ± 0.01^{C,d}	12.82 ± 0.02^{D,f}
AA + H_2O_2 (%2) + Na	20.08 ± 0.02^{A,j}	14.50 ± 0.01^{B,m}	13.88 ± 0.02^{C,i}	13.02 ± 0.03^{D,e}
CA + H_2O_2 (%2) + Na	23.66 ± 0.02^{A,g}	17.96 ± 0.01^{B,e}	14.55 ± 0.01^{C,g}	11.01 ± 0.02^{D,k}

The values are the means ± SD of triplicate experiments. Means followed by different uppercase and lowercase letters indicate significant differences in row and column, respectively ($P < 0.05$).

the concentration of ions, such as Na^+ and Cl^-, as well as changing the temperature of the sanitizing treatment, can affect the permeability of the cell membrane, and thus would cause variations in the rate of exchange among intracellular compounds with the surrounding environment. A synergic effect was observed by the LA + NaCl treatment, so that the highest ascorbic acid content in treated samples was achieved (26.55 ± 0.02–16.82 ± 0.01 mg/100 g) in all of the evaluation times. In this context the negative effects were observed when NaCl was added to AA and CA sanitizers ($P < 0.05$). Results indicated that the addition of NaCl (%7) to sanitizers containing organic acid and hydrogen peroxide (except at first and 2th days for LA + H_2O_2 (1%) + NaCl and CA + H_2O_2 (%1) + NaCl at first day) showed an incremental effect on the save of ascorbic acid content ($P < 0.05$). On the other hand, changes in the concentration of H_2O_2 in the ternary combination were followed by changes to the protective effect on ascorbic acid. Accordingly, the LA + H_2O_2 (2%) + NaCl, AA + H_2O_2 (1%) + NaCl and CA + H_2O_2 (1%) + NaCl were more effective in protecting ascorbic acid, compared to the LA + H_2O_2 (1%) + NaCl, AA + H_2O_2 (2%) + NaCl and CA + H_2O_2 (2%) + NaCl, respectively ($P < 0.05$).

Kenny and O'Beirne (2009) stated that disinfecting the lettuce with chlorinated water reduced the amount of vitamin C by 25% during 8 days of storage at 4 °C, compared to dipping the samples in water. These results indicate that the amount of vitamin C can be affected by the chemical composition and concentration of the disinfectant solution and the duration of storage.

3.3. Total phenolics

Phenolic compounds may comprise phytochemicals, and they constitute the major cause of antioxidative properties in vegetables and plant foods (Cartea et al., 2011). The results (Table 3) showed that the total phenolic content of the samples decreased over the storage time ($P < 0.05$). The decrease in total phenol content during storage at 4 °C in other plants has also been reported by Galani et al. (2017). In contrast, Kenny and O'Beirne (2009) reported that the total amount of phenolics did not change significantly in iceberg lettuce when it was washed with chlorinated water and distilled water during 8 days of storage at 4 °C. Cellular metabolism in vegetable tissues can be affected by the disinfectant solution (Roura et al., 2008) which can also change total phenolic compounds as the activity levels of polyphenol-oxidase enzymes and those of phenylalanine ammonia-lyase (PAL) can be influenced in response to the disinfectant (Galani et al., 2017).

The highest total phenolic content was observed in control *a* (112.2 ± 0.5 mg GAE/100 g), and by CA + H_2O_2 (2%) on the 2th day (56.6 ± 0.4 mg GAE/100 g) and also by LA + H_2O_2 (2%) on the 3th and 4th days (42.4 ± 0.2 and 40.2 ± 0.2 mg GAE/100 g, respectively) ($P < 0.05$). The comparison of total phenolic contents in organic acid sanitizers showed that only LA (18.10–49.62%) had a higher protective effect compared to control b ($P < 0.05$). Table 3 shows that the increase in H_2O_2 concentration from 1 to 2% in the H_2O_2 sanitizer leads to a decrease in total phenol content ($P < 0.05$) (see Section 3.2). The oxidation and subsequent degradation of phenolic compounds can be due to the decomposition of H_2O_2 products (Sapers et al., 1999; Sapers and Simmons, 1998). In fact, De et al. (1999) reported that a main reactive species that can dissociate the benzene ring in phenolic compounds is the °OH radical, which can also break the substrate into CO_2 and H_2O. Increasing the concentration of H_2O_2 from 1 to 2% in combination sanitizers of organic acid + H_2O_2 was monitored to increase the total phenol content in the samples. Sanitizing the basil leaves with NaCl (7%) reduced the amount of total phenol in basil samples when compared to control samples at first and 2th day ($P < 0.05$). Adding of NaCl (7%) to organic acid sanitizers reduced the total phenolic content when the treatments LA + NaCl, CA + NaCl (except at 1 day) and AA + NaCl (except at 3 and 4) ($P < 0.05$) were used. Also trinary combination treatment showed a different effect on the total content of phenolics (see Section 3.2).

3.4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The most important mechanisms of antioxidants are the stabilization of free radicals which can have many negative effects on human health (Cartea et al., 2011). DPPH (1,1-diphenyl-2-picrylhydrazyl) is an indicator of radical scavenging activity among extracts (Cotelle et al., 1996). Results indicated (Table 4) that, over time, the scavenging

Table 3
Effect of different sanitizing treatments on total phenolic content (mg GAE/100 g fresh wt. of sample) in basil leaves, during storage time at (4 ± 1 °C).

Treatment	Time (day)			
	1	2	3	4
Control *a*	112.2 ± 0.5^{A,a}	37.5 ± 0.5^{B,j}	25.0 ± 0.4^{C,j}	19.4 ± 0.3^{D,h}
Control *b*	66.4 ± 0.9^{A,g}	34.0 ± 0.3^{B,l}	26.2 ± 0.3^{C,i}	21.8 ± 0.4^{D,g}
LA	79.0 ± 0.2^{A,f}	53.3 ± 0.4^{B,b}	39.3 ± 0.2^{C,b}	31.7 ± 0.3^{D,c}
AA	56.0 ± 0.4^{A,n}	21.5 ± 0.4^{B,r}	15.3 ± 0.2^{C,p}	11.7 ± 0.2^{D,n}
CA	65.2 ± 0.2^{A,h}	32.4 ± 0.5^{B,m}	24.2 ± 0.2^{C,k}	19.4 ± 0.2^{D,h}
Na	64.4 ± 0.3^{A,i}	27.1 ± 0.4^{C,o}	36.8 ± 0.6^{B,d}	21.4 ± 0.2^{D,g}
H_2O_2 (1%)	60.1 ± 0.6^{A,l}	51.0 ± 0.3^{B,c}	29.6 ± 0.3^{C,g}	16.4 ± 0.1^{D,d}
H_2O_2 (2%)	53.5 ± 0.2^{o}	48.1 ± 0.1^{B,d}	26.2 ± 0.4^{C,i}	15.2 ± 0.1^{D,l}
LA + H_2O_2 (%1)	59.0 ± 0.1^{A,m}	50.9 ± 0.2^{B,c}	38.0 ± 0.1^{C,b}	31.4 ± 0.1^{D,c}
AA + H_2O_2 (%1)	48.5 ± 0.6^{A,q}	15.3 ± 0.3^{B,s}	12.9 ± 0.1^{C,q}	11.8 ± 0.2^{D,n}
CA + H_2O_2 (%1)	50.8 ± 0.4^{A,p}	34.8 ± 0.4^{B,k}	29.0 ± 0.2^{C,h}	28.1 ± 0.4^{D,e}
LA + H_2O_2 (%2)	63.4 ± 0.4^{A,j}	51.5 ± 0.4^{B,c}	42.4 ± 0.2^{C,a}	40.2 ± 0.22^{D,a}
AA + H_2O_2 (%2)	59.5 ± 0.2^{A,lm}	39.0 ± 0.5^{B,i}	34.5 ± 0.3^{C,e}	32.6 ± 0.3^{D,b}
CA + H_2O_2 (%2)	109.3 ± 0.4^{A,b}	56.6 ± 0.4^{B,a}	34.7 ± 0.4^{A,e}	30.3 ± 0.4^{D,d}
LA + Na	65.3 ± 0.2^{A,h}	40.1 ± 0.3^{B,h}	31.0 ± 0.1^{C,f}	27.1 ± 0.3^{D,f}
AA + Na	51.2 ± 0.3^{A,p}	21.5 ± 0.4^{B,r}	17.4 ± 0.2^{C,n}	15.7 ± 0.3^{D,k}
CA + Na	105.0 ± 0.3^{A,c}	28.0 ± 0.3^{B,n}	21.9 ± 0.3^{C,l}	17.9 ± 0.3^{D,i}
LA + H_2O_2 (%1) + Na	78.5 ± 0.2^{A,f}	45.3 ± 0.3^{B,e}	35.0 ± 0.1^{C,e}	30.3 ± 0.3^{D,d}
AA + H_2O_2 (%1) + Na	37.0 ± 0.4^{A,s}	13.8 ± 0.2^{B,t}	10.6 ± 0.2^{C,r}	8.4 ± 0.2^{D,p}
CA + H_2O_2 (%1) + Na	62.6 ± 0.2^{A,k}	42.1 ± 0.1^{B,g}	28.7 ± 0.2^{C,h}	19.7 ± 0.4^{D,h}
LA + H_2O_2 (%2) + Na	44.9 ± 0.3^{A,r}	24.8 ± 0.3^{B,p}	20.3 ± 0.7^{C,m}	13.3 ± 0.3^{D,m}
AA + H_2O_2 (%2) + Na	80.6 ± 0.2^{A,e}	23.1 ± 0.4^{B,q}	16.7 ± 0.3^{C,o}	9.5 ± 0.2^{D,o}
CA + H_2O_2 (%2) + Na	94.3 ± 0.3^{A,d}	43.7 ± 0.3^{B,f}	30.5 ± 0.2^{C,f}	18.1 ± 0.3^{D,i}

The values are the means ± SD of triplicate experiments. Means followed by different uppercase and lowercase letters indicate significant differences in row and column, respectively ($P < 0.05$).

activity decreased in all samples ($P < 0.05$). These results confirm previous reports by Farajvand et al. (2015). The highest percentage of scavenging activity was observed in control *a* which involved no washing (95.2 ± 0.5% − 90.1 ± 0.6%) on the first and 2th day of chilled storage times and by LA + H_2O_2 (1%) (77.2 ± 0.2 − 73.3 ± 0.1) on the third and fourth days ($P < 0.05$). The results of this study showed that changes in DPPH values with total phenolic compounds ($R^2 = 0.84$) had a stronger correlation with ascorbic acid values ($R^2 = 0.65$) which is consistent with previous results (Kenny and O'Beirne, 2009; Rahman et al., 2011).

3.5. Overall acceptability

Sensory evaluation is one of the most important examinations on quality of food and their processes. Fig. 1(a, b, c) shows that all samples showed an overall acceptance score ≥ 5.0 on the first day, but the score decreased during the cold storage period. This is probably due to the breakdown of the aroma compounds and the removal of moisture from

Table 4
Effect of different sanitizing treatments on DPPH· radical scavenging activity (%) in basil leaves, during storage time at (4 ± 1 °C).

Treatment	Time (day)			
	1	2	3	4
Control *a*	95.2 ± 0.5^{A,a}	90.1 ± 0.6^{B,a}	47.3 ± 0.9^{C,r}	34.6 ± 0.7^{D,q}
Control *b*	88.5 ± 0.2^{A,i}	86.3 ± 0.3^{B,b}	56.5 ± 0.2^{C,m}	20.5 ± 0.3^{D,s}
LA	88.7 ± 0.4^{A,hi}	69.9 ± 0.3^{B,h}	57.1 ± 0.2^{C,l}	39.4 ± 0.2^{D,p}
AA	89.1 ± 0.3^{A,fgh}	74.9 ± 0.2^{B,f}	60.7 ± 0.8^{C,j}	52.5 ± 0.2^{D,i}
CA	83.3 ± 0.1^{A,m}	69.0 ± 0.1^{B,i}	45.3 ± 0.3^{C,s}	25.2 ± 1.5^{D,r}
Na	84.4 ± 0.1^{A,l}	61.9 ± 0.3^{B,m}	49.7 ± 0.2^{C,q}	51.5 ± 0.2^{D,j}
H_2O_2 (1%)	83.4 ± 0.3^{A,m}	66.9 ± 0.2^{B,i}	56.7 ± 0.2^{C,lm}	48.5 ± 0.1^{D,k}
H_2O_2 (2%)	81.2 ± 0.1^{A,n}	63.6 ± 0.1^{B,m}	51.7 ± 0.3^{C,p}	44.5 ± 0.2^{D,m}
LA + H_2O_2 (%1)	89.6 ± 0.1^{A,f}	78.1 ± 0.2^{B,e}	73.3 ± 0.2^{C,e}	68.9 ± 0.2^{D,d}
AA + H_2O_2 (%1)	94.1 ± 0.6^{A,b}	82.0 ± 0.1^{B,c}	77.2 ± 0.2^{C,a}	73.3 ± 0.1^{D,a}
CA + H_2O_2 (%1)	89.4 ± 0.2^{A,l}	70.1 ± 0.2^{B,hi}	55.0 ± 0.1^{C,n}	40.7 ± 0.2^{D,o}
LA + H_2O_2 (%2)	89.0 ± 0.3^{A,ghi}	73.6 ± 0.3^{B,g}	65.0 ± 0.2^{C,g}	58.0 ± 0.3^{D,f}
AA + H_2O_2 (%2)	91.1 ± 0.3^{A,c}	79.6 ± 0.1^{B,d}	74.5 ± 0.1^{C,d}	69.6 ± 0.3^{D,c}
CA + H_2O_2 (%2)	90.1 ± 0.2^{A,e}	81.8 ± 0.4^{B,c}	64.6 ± 0.2^{C,g}	47.4 ± 0.3^{D,l}
LA + Na	91.2 ± 0.2^{A,c}	79.3 ± 0.6^{B,d}	75.2 ± 0.2^{C,c}	70.6 ± 0.1^{D,b}
AA + Na	90.6 ± 0.4^{A,d}	81.6 ± 0.3^{B,c}	76.3 ± 0.3^{C,b}	70.6 ± 0.3^{D,b}
CA + Na	89.6 ± 0.4^{A,f}	73.5 ± 0.3^{B,g}	64.0 ± 0.1^{C,h}	54.6 ± 0.2^{D,h}
LA + H_2O_2 (%1) + Na	89.3 ± 0.1^{A,fg}	68.9 ± 0.4^{B,i}	60.5 ± 0.3^{C,j}	51.3 ± 0.3^{D,j}
AA + H_2O_2 (%1) + Na	89.4 ± 0.1^{A,fg}	74.4 ± 0.3^{B,f}	66.4 ± 0.3^{C,f}	59.0 ± 0.3^{D,e}
CA + H_2O_2 (%1) + Na	84.4 ± 0.1^{A,l}	68.9 ± 0.2^{B,i}	54.7 ± 0.2^{C,n}	40.5 ± 0.2^{D,o}
LA + H_2O_2 (%2) + Na	88.5 ± 0.2^{A,i}	70.3 ± 0.2^{B,h}	63.2 ± 0.2^{C,i}	56.9 ± 0.3^{D,g}
AA + H_2O_2 (%2) + Na	87.8 ± 0.1^{A,j}	73.4 ± 0.3^{B,g}	58.3 ± 0.3^{C,k}	42.4 ± 0.2^{D,n}
CA + H_2O_2 (%2) + Na	85.7 ± 0.3^{A,k}	65.5 ± 0.4^{B,k}	53.2 ± 0.2^{C,o}	40.4 ± 0.1^{D,o}

The values are the means ± SD of triplicate experiments. Means followed by different uppercase and lowercase letters indicate significant differences in row and column, respectively ($P < 0.05$).

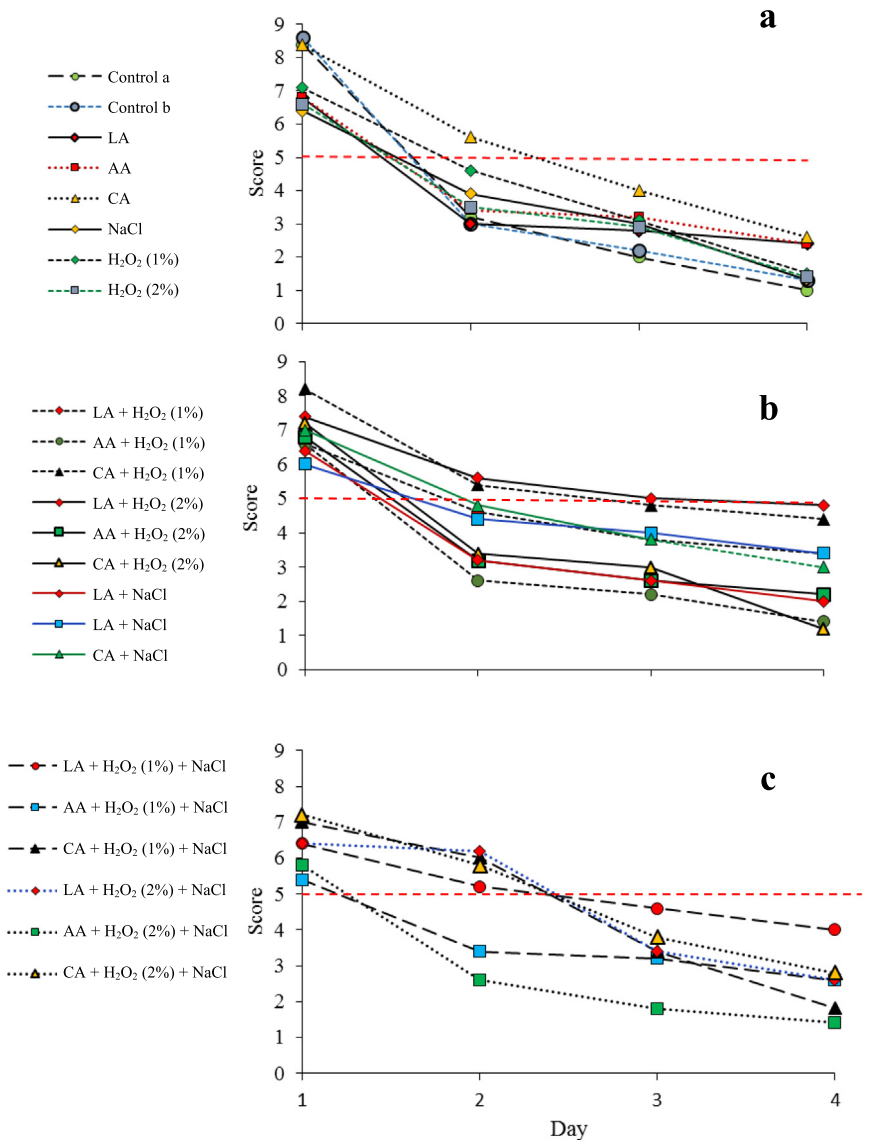

Fig. 1. Effect of different sanitizing treatments on overall acceptability of basil leave. Columns represent mean values ± SD.

the tissue during cold storage (Rico et al., 2007). On the other hand, the enzymatic activities of agents such as lipo-oxygenase, PPO and PAL, as well as the growth and activity of microorganisms, can decrease the quality of vegetables (Lin et al., 2002; Rico et al., 2007; Roura et al., 2008). As Fig. 1a and b shows, overall acceptance scores higher than 8.0 were observed in control a, control b, CA and CA + H_2O_2 (1%) samples on the first day. Rahman et al. (2011) reported that adding citric acid to a solution of alkaline-electrolyzed water can improve the sensory qualities of carrots.

The highest overall acceptance score at second day was obtained for treatment LA + H_2O_2 (2%) + NaCl (6.2 ± 0.8), and at third day for LA + H_2O_2 (2%) (5.0 ± 0.7). However, none of the samples were able to show scores higher than 5 on the fourth day. Adding different concentrations of hydrogen peroxide to organic acid sanitizers showed variable effects on the overall acceptance of basil leaves. The decrease in overall acceptance score of samples occurred by adding NaCl (7%) to CA sanitizer, especially on the second day. But adding NaCl (7%) to sanitizers containing CA + H_2O_2 (2%) increased the overall acceptance score (by 0.0 to 133%). Precise information is not available on how the chemical compounds of the treatments affect the sensory properties of the leaves. The results of this study and those of previous research show that the sensory properties of leafy vegetables are influenced by chemical compounds, the type of treatment on the metabolic activity of cells, plant tissue properties, microbial population and the duration of the storage period (Dewhirst et al., 2017; Rahman et al., 2011; Rico et al., 2007;).

4. Conclusion

Results from the present study clearly show that sanitizing treatments including lactic, acetic and citric acids (2%), NaCl (7%) and H_2O_2 (1 and 2%), either when used alone or in combinations, and when coupled with mild heat (40 °C), can reduce the impact of *E. coli* O157: H7 efficiently. However, negative effects of sanitizing treatments were observed on ascorbic acid, total phenolics content and scavenging activity in basil leaves beginning from the first day of treatments, when compared with unwashed treatment (control *a*). Different chemical treatments showed different protective effects on the nutritional content and sensorial properties of basil leaves were affected by the type and concentrations of sanitizer components. Based on the anti-pathogenic activities and the lower limit of overall appearance score of samples (≥5), LA + H_2O_2 (2%) treatment introduced for sanitizing treatment. Since the highest nutritional and sensory characteristics of treatments were observed immediately after sanitization, we

recommend that it is best to prepare vegetables on a daily basis and to consume them without any substantial delays of > 24 h after sanitization. Given the reasonable results of this study, it is recommended that future instances of research consider different strains of pathogenic bacteria in the context of such types of experiments.

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