SUPERIOR EFFICACY OF DISINFECTANT WASH SOLUTION OF CITRIC ACID COMBINED WITH OTHER GENERALLY RECOGNIZED AS SAFE COMPOUNDS AGAINST FOODBORNE PATHOGEN *ESCHERICHIA COLI* O157:H7

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Abstract. *Escherichia coli* O157:H7 is the etiological agent of severe intestinal infection in humans causing foodborne illness through consumption of contaminated raw food. The study tested bactericidal activity against *E. coli* O157:H7 of citric acid solution alone or in combination with three other generally recognized as safe (GRAS) compounds, four common household wash solutions and a commercial wash solution. Citric acid solution (0.112 mg/ml) was more effective, achieving 1, 6 and 12 $\log_{10}$ reduction in 3, 19 and 38 minutes, respectively compared to a commercial wash solution, with 1 $\log_{10}$ reduction in 15 minutes. Bactericidal efficacy of the citric acid solution was highly improved when supplemented with GRAS compounds, namely, ethylenediaminetetraacetic acid (0.01 g/ml), L-ascorbic acid (0.013 g/ml) and sodium lauryl ether sulfate (0.031 g/ml), at 4°C and 25°C, with >3 $\log_{10}$ reduction in 5 minutes; the efficacy was slightly reduced at 40°C. However, household wash solutions (distilled water, 0.04% (v/v) hydrogen peroxide, 20.0% (w/v) sodium chloride, and 2.50% (v/v) vinegar) were markedly less effective, with 1 $\log_{10}$ reduction in 259-1,550 minutes. These findings indicate a solution of citric acid together with other GRAS compounds have the potential as a superior disinfectant or preservative in inhibiting *E. coli* O157:H7 growth compared to common household and commercial disinfectant wash solutions. This study is critically relevant to ongoing strategies in diminishing the risk of coliform contamination in products.

Keywords: *Escherichia coli* O157:H7, antimicrobial efficacy, citric acid, foodborne illness, generally recognized as safe (GRAS) compound

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INTRODUCTION

Food safety is a critical health, economic and social issue worldwide because millions of people are affected by foodborne illnesses (European Commission, 2002; Barker-Reid *et al*, 2009). It is estimated that 2.2 million people in the world die annually due to food- and waterborne diseases (WHO, 2002). In USA, 9.4 million serious food-related illnesses occur every year (Scallan *et al*, 2011) and in the Southeast Asian region, foodborne diseases stemming from pathogenic *Escherichia coli*, non-typhoidal *Salmonella* and noroviruses cause morbidity among more than 150 million people and a mortality of 175,000 annually (WHO, 2015). These pathogens are usually considered as public health risk factors in developed and developing countries (WHO, n.d.). Aside from traceability in the food chain, controllability is also important for ensuring consumer safety and protecting foods from hazards at the point of consumption.

E. coli O157:H7, a Shiga-toxin-producing *E. coli* (STEC) strain, is considered a food contaminant of concern, which can cause serious foodborne disease in humans (Mead *et al*, 1999). Outbreaks of *E. coli* O157:H7 have mostly been associated with the consumption of fecal contaminated food, such as undercooked meat products, unpasteurized milk and salads (Feng, 2014; Herman *et al*, 2015). Furthermore, a person-to-person transmission is also possible by close contact within families or at schools and in hospital settings (Busani *et al*, 2006). Infection with as little as 1,000 STEC cells can cause severe diarrhea, hemolytic uremic syndrome, hemorrhagic colitis, and thrombotic thrombocytopenic purpura (Karmali, 2009). Nearly 1.7 billion cases of STEC-associated diarrheal disease are recorded annually worldwide (WHO, 2017). Following pneumonia, it is the second leading cause of death in children below 5 years of age (approximately 760,000 each year) (Chowdhury *et al*, 2015).

E. coli O157:H7 can grow over a broad temperature range and in acidic condition (Chaucheyras-Durand *et al*, 2010). The degree of bacterial susceptibility to low pH varies among pathogens (Uljas and Ingham, 1998; Waterman and Small, 1998) depending on their ability to protect against acid stress (Bae and Lee, 2017). *E. coli* O157:H7 contains an efficient proton efflux system (Cash *et al*, 1974).

One method in safeguarding against bacterial infections is through the use of a hurdle effect technology (HET) (Chapman and Ross, 2009), which combines the application of several protective methods (either physical or chemical in nature) that are regarded as having bactericidal or bacteriostatic effects (Walkling-Ribeiro *et al*, 2011; Narayanan *et al*, 2013; Singh and Shalini, 2016; Munoz *et al*, 2017). Disturbance of microorganism homeostasis is a critical principle of food preservation, but microbial stress reactions may compromise food preservation, while, on the other hand, metabolic exhaustion of microorganisms in HET-processed food or food products fosters food preservation (Singh and Shalini, 2016).

In general, the primary targets of antimicrobials are cell wall, plasma membrane and certain metabolic pathways associated with replication and protein translation (Ricke, 2003). Citric acid, an organic acid, is considered as a generally recognized as safe (GRAS) product and a natural antimicrobial with the ability to repress or inactivate pathogenic microbes (Akbas and Olmez, 2007). Although *E. coli* O157:H7 cells can develop acid resistance, organic acids are still effective as antimicrobials due to the ability of their undissociated forms to pass through bacterial plasma membrane and release protons within the cell (Hosein *et al*, 2011), thereby interrupting microbial acid-base homeostasis (Gamlath *et al*, 2004; Barwal *et al*, 2005; Vibhakara *et al*, 2006).

Here, growth inhibition of *E. coli* O157:H7 isolated from lettuce obtained from Divisoria fresh market, National Capital Region, Philippines, was evaluated against household and commercial wash solutions and citric acid alone or in combination with other GRAS compounds. The findings should provide information regarding application of GRAS compounds as disinfectants and preservatives in food products to assist in reducing risk of microbial contamination and food poisoning.

MATERIALS AND METHODS

Culture and molecular identification of *E. coli* O157:H7

Five g of fresh lettuce leaves obtained from Divisoria fresh market, National Capital Region (NCR), Philippines (in October 2015) were mixed in 40 ml of 0.1% buffered peptone water (HiMedia Laboratories Inc, Kennett Square, PA) and a ten-fold serial dilutions were prepared, which were then passed through 0.45 µm filter, plated on thermotolerant *E. coli* Difco™ modified mTEC agar (BD Difco Laboratories, Sparks, MD) and initially incubated at 35°C for 2 hours and then at 44.5°C for 24 hours (US EPA, 2005). Blue to deep blue bacterial colonies were streaked on eosin methylene blue (EMB) agar

(BD Difco Lab.) and incubated at 35°C for 24 hours. Colonies with green metallic sheen and dark center were considered as putative thermotolerant *E. coli* (US EPA, 2005).

Genomic DNA was extracted using a boil-lysis method (Vital *et al*, 2019). Bacterial 16S rDNA was amplified used the universal primers 27F (5′-AGAGTTTGATCATGG CTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Turner *et al*, 1999). Polymerase chain reaction (PCR) mixture contained 10^{-11}-10^{-6} g/µl DNA template, 2X Green Mastermix (Promega, Madison, WI), 10 µM of each primer, and nuclease-free water to make a final volume of 20 µl. Thermocycling was carried out using Bio-Rad MyCycler™ Thermal Cycler (Bio-Rad, Hercules, CA) as follows: 94°C for 3 minutes; 35 cycles of 94°C for 30 seconds, 60°C for 60 seconds and 68°C for 120 seconds; and a final step of 68°C for 7 minutes. Amplicons were analyzed by 1.50% agarose gel-electrophoresis, visualized using Invitrogen™ SYBR™ Safe DNA Gel Stain (ThermoFisher, Waltham, MA), gel extracted and directly sequenced (MACROGEN, Seoul, Korea). Sequences were aligned using a BioEdit Software 7.0.5.3 (https://bioedit.software.informer.com/7.0/) and analyzed by a Basic Local Alignment Search Tool (BLAST) software (https://blast.ncbi.nlm.nih.gov).

Preparation of bacterial suspension

Thermotolerant *E. coli* O157:H7 isolate (GenBank accession no. CP009685.1) was cultured in 1.0 ml of BBLTM TrypticaseTM Soy Broth (TSB) (Becton, Dickinson and Co, Sparks, France) at 37°C for 24 hours and centrifuged at 1000 g for 15 minutes. Pellet was washed twice with 0.10 % peptone water (HiMedia Laboratories Inc, Kennett Square, PA) and suspended in 0.10% peptone water (Kim and Rhee, 2015). *E. coli* O157:H7 standard from the Biological Research and Services Laboratory (Quezon City, National Capital Region, Philippines) was used as positive control. For assay of solutions of GRAS compounds *E. coli* O157:H7 suspension was adjusted to 0.5 McFarland standard ($1.5x10^8$ CFU/ml) and $A_{600\ nm}$ adjusted to $5.0x10^5$ CFU/ml (Elshikh *et al*, 2016).

Preparation of test solutions

Test solutions were citric acid (CA) (0.186 mg/ml), a commercial wash solution [0.08% (v/v)], distilled water, L-ascorbic acid (0.10 g/ml), ethylenediaminetetraacetic acid (EDTA) (0.08 g/ml), hydrogen peroxide [0.04% (v/v)], sodium chloride [20.0% (w/v)], sodium lauryl ether sulfate (SLS) (0.50 g/ml)], and white vinegar [2.50% (v/v)]. A four-component test solution contained 0.013 g/ml L-ascorbic acid, 0.112 mg/ml CA, 0.01 g/ml EDTA, and 0.031 g/ml SLS, and adjusted to pH 3.0 with 1.0 M hydrochloric acid (HCl).

Decimal reduction time (D-)value determination

Bactericidal effect of test solutions was expressed in terms of D-value, which is defined as the exposure time required, under a specific set of conditions, to result in 10^{-1} initial bacterial population (*ie* 90% reduction in initial population) (Mazzola *et al*, 2003). D-value is calculated from the following equation (Baranyi and Roberts, 1994):

$$\text{D-value} = t/n \qquad (1)$$

where t = exposure time (minute)
n = 10^{-1} initial population

Estimated confidence level and final number of surviving bacteria/ml solution are expressed as D- value at 10^{-6} and 10^{-12} initial population calculated as 6x and 12x D-value respectively. Extent of treatment for disinfection of test solution is determined by a predicted time for microorganism to be reduced to 10^{-6} and 10^{-12} initial population, which is equivalent to 6x and 12x D-value respectively using the following equation (Mazzola *et al*, 2003).

Experimentally, a D-value is determined from the negative reciprocal of the slope of a linear portion of a plot of $\log_{10}$ CFU/ml versus time of exposure to a wash solution at given temperature (Mazzola *et al*, 2003), calculated using an Excel DMFit 3.5 spreadsheet, with R^2 value (0-1.00) as a measurement of goodness-of-fit of curve (1.00 being perfect fitness) (Baranyi and Roberts, 1994).

Optimal CA solution D-value determination

A 0.5 McFarland standard of *E. coli* O157:H7 suspension was suspended in 10-fold serial dilutions of a CA stock solution (0.186 mg/ml), and 100-µl aliquot of each bacterial suspension was plated on Nutrient Agar (NA) (HiMedia Laboratories Pvt Ltd, Mumbai, India), incubated at 37°C for 24 hours and remaining bacteria counted. Each experiment was performed in duplicate and result expressed as $\log_{10}$ mean CFU/ml. The solution producing the lowest D-value in comparison to a commercial wash solution was chosen for further studies.

Resazurin-based microtiter assay for determining bactericidal effectiveness of GRAS compounds

Resazurin (Merck Inc, Taguig City, Philippines) (0.015% (w/v) in water) was filtered (0.22 µm pore) and stored at 4°C for use within 2 weeks. GRAS chemicals (L-ascorbic acid, EDTA and SLS) were dissolved in Mueller Hinton Broth (MHB) (HiMedia Laboratories Inc, Kennett Square, PA) and 2-fold serially diluted to twice the concentration of the final test. A 100-µl aliquot of GRAS solutions was dispensed into wells of a 96-well microtiter plate (ThermoFisher, Waltham, MA). Control wells contained

100 µl of a standard inoculum in MHB (positive control) and 100 µl of MHB only (negative control). Plates were incubated at 37°C for 24 hours, then 15-µl aliquot of resazurin solution was added to each well and plate incubated in the dark for 2-4 hours for observation of color change (from blue to pink). Estimated minimum inhibitory concentration (MIC) (lowest concentration of a chemical that suppresses visible growth of an organism after incubation) and minimum bactericidal concentration (MBC) (lowest concentration of a chemical that reduces bacteria to 10^{-3} original population (99.9% reduction) within a given period of time) were determined (Andrews, 2001). Percent reduction and log reduction were calculated as follows:

$$\text{Percent reduction} = [(A-B)/A] \times 100 \quad (2)$$

$$\text{Log}_{10}\text{ reduction} = \text{Log}_{10}(\text{Log}_{10} A) - \text{Log}_{10}(\text{Log}_{10} B) \quad (3)$$

where A = Number of viable cells before treatment
B = Number of viable cells after treatment

Data analysis

Value is expressed as mean ± standard deviation (SD) and mean CFU/ml are log-transformed to improve normality and homoscedasticity. Results of growth inhibition parameters are analyzed by one way analysis of variance (ANOVA) and Student's t-test using PAST version 4.03 statistical software (https://past.en.lo4d/download). A *p*-value <0.05 is considered statistically significant.

RESULTS

In order to determine the most appropriate CA solution for comparison with other wash solutions and combination solution studies, CA solutions (n = 13) of different concentrations (0.050-0.186 mg/ml) and acidic pH (2.1-2.4) were analyzed for their bactericidal effectiveness against *E. coli* O157:H7 compared to a commercial wash solution. The most effective was CA 1 solution (0.112 mg/ml) with a D-value of 3.1 ± 0.2 minutes compared to that of a commercial wash solution (15 ± 2 minutes) (Table 1). Predicted inactivation exposure time of CA 1 solution to attain 10^{-6} and 10^{-12} original population was 19 and 38 minutes respectively compared to a commercial wash solution of 92 and 185 minutes respectively.

Bactericidal effects of three GRAS chemicals, namely, L-ascorbic acid, EDTA and SLS, were assessed against

Table 1

Bactericidal effects of different citric acid concentrations, commercial wash, and household solutions against *Escherichia coli* O157:H7 at 25°C

Solution	pH	Concentration (mg/ml)	D-value (minute) Mean ± SD (n = 4)	Total exposure time (t) (minute)[†]		$R^{2‡}$ ± SD
				t^a	t^b	
Citric acid (CA)						
1	2.2	0.112	3.1 ± 0.2*	19	38	0.98 ± 0.06
2	2.3	0.088	9.2 ± 0.1*	55	110	0.88 ± 0.00
3	2.3	0.092	8 ± 1*	48	95	0.75 ± 0.04
4	2.3	0.086	13.6 ± 0.3	81	163	0.90 ± 0.01
5	2.2	0.153	10.0 ± 0.1	60	120	0.92 ± 0.02
6	2.2	0.119	4.4 ± 0.5*	26	53	0.98 ± 0.00
7	2.2	0.120	11.5 ± 0.3*	69	138	0.74 ± 0.03
8	2.1	0.186	13.1 ± 0.0	79	157	0.89 ± 0.00
9	2.2	0.155	6.2 ± 0.8*	37	74	0.94 ± 0.01
10	2.4	0.050	7.7 ± 0.6*	46	92	0.80 ± 0.05
11	2.4	0.060	4.0 ± 0.4*	24	48	0.99 ± 0.00
12	2.3	0.095	17 ± 1	100	201	0.97 ± 0.01

Table 1 (cont)

Solution	pH	Concentration (mg/ml)	D-value (minute) Mean ± SD (n = 4)	Total exposure time (t) (minute)†		$R^{2‡}$ ± SD
				t^a	t^b	
Commercial wash (Brand XY)	5.7	0.08% (v/v)	15 ± 2	92	185	0.89 ± 0.02
Vinegar	2.7	2.50	259.04 ± 34.59	259	1550	0.26 ± 0.07
Hydrogen peroxide	5.0	0.04	382.76 ± 9.86	259	1550	0.21 ± 0.02
Sodium chloride	7.2	20.00	487.86 ± 70.79	259	1550	0.02 ± 0.00
Distilled water	6.8	No value	1549.77 ± 277.40	259	1550	0.30 ± 0.08

*p-value<0.05 compared to commercial wash solution is statistically significant

†t^a: Predicted inactivation exposure time to attain 10^{-6} original population calculated from 6x D-value; t^b: Predicted inactivation exposure time to attain 10^{-12} original population from 12x D-value

‡Measurement of goodness-of-fit of curve (Baranyi and Roberts, 1994)

D-value: Exposure time required to attain 90% reduction of initial population; mg/ml: milligram per milliliter; NA: not applicable; R^2: coefficient of determination; SD: standard deviation; v/v: volume per volume; w/v: weight per volume

E. coli O157:H7 using a resazurin method. L-ascorbic acid and EDTA solutions had similar minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), both values being 3 folds better than SLS solution (Table 2). The two former GRAS compounds exhibited 100% growth reduction and >2.5 log growth reduction compared to that of ~80% and ~0.8 respectively for SLS solution.

When CA 1 solution was included with the three GRAS compounds to form a four-component wash solution (0.013 g/ml L-ascorbic acid, 0.112 g/l CA, 0.01 g/ml EDTA, and 0.031 g/ml SLS, adjusted to pH 3.0), 99.9% killing of *E. coli* O157:H7 was achieved following exposure of 5 minutes at 25°C and even at 4°C, although the bactericidal effect was slightly reduced a higher temperature (40°C) (Table 3). These findings, when compared to the D-value (3 minutes) of CA 1 solution alone (Table 1), clearly demonstrated the augmentation of CA bactericidal property by the presence of the three GRAS compounds. This observation was even more apparent when D-values of four household wash solutions, namely, distilled water, 0.04% (v/v) hydrogen peroxide, 20.0% (w/v) sodium chloride, and 2.50% (v/v) vinegar, were measured: range 259-1,550 minutes (Table 1). Moreover, their R^2 values were ≤0.30.

DISCUSSION

Several studies have been published on CA antimicrobial effect of citric acid. Sorrells *et al* (1989) reported that CA possesses higher antimicrobial property compared to acetic and lactic acid. Oulkheir *et al* (2015) noted bactericidal activity against *E. coli* reached 6.3 $\log_{10}$ reduction when treated with a citric acid-supplemented tryptic soy broth. In addition to *E. coli*, citric acid shows better antimicrobial activity compared to other organic acids at equimolar concentrations against other pathogens, *eg Yersinia enterocolitica* (Brackett, 1987) and *Listeria monocytogenes* (Buchanan and Golden, 1994).

Nevertheless, it should be noted that implementation of organic acids as antimicrobial agents depends on several characteristics of the acids, such as chemical structure, physical property, molecular weight, pK_a value, inhibitory concentration, exposure time and type of the target microbes (Thompson and Hinton, 1997; Davidson *et al*, 2005). A weak acid (pK_a 3-5) is preferred over a stronger one as the former is better able to penetrate a cell membrane, as well as having <7 carbon atoms (Davidson *et al*, 2005; Shahidi *et al*, 2014). Hence, citric acid ($C_6H_8O_7$; pK_a of 4.76) is regarded as a suitable organic acid of choice.

Itelima and Agina (2014) demonstrated that dipping of meat and carrot for up to 40 minutes in CA solutions at pH of 3.0, 3.8 and 4.6

Table 2

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), percent growth reduction, and log growth reduction of *Escherichia coli* O157:H7 treated with solutions of generally regarded as safe (GRAS) chemicals

GRAS compound	MIC (g/ml)	MBC (g/ml)	Percent growth reduction Mean ± SD (n = 3)	Log_{10} growth reduction Mean ± SD (n = 3)
SLS	0.031	0.063	81 ± 6	0.8 ± 0.2
L-ascorbic acid	0.013	0.025	100 ± 0*	>3.0 ± 0.02*
EDTA	0.010	0.020	100 ± 0.1*	2.5 ± 0.1*

*p-value <0.05 compared to SLS

EDTA: ethylenediaminetetraacetic acid; g/ml: gram per milliliter; SD: standard deviation; SLS: sodium lauryl ether sulfate

Growth of *E. coli* after 24 hours at 37°C was measured using a resazurin assay.

Table 3

Percent growth reduction and log growth reduction of *Escherichia coli* O157:H7 following a 5-minute exposure at different temperatures to a four-component wash solution

Temperature (°C)	Percent growth reduction Mean ± SD (n = 3)	Log growth reduction Mean ± SD (n = 3)
4	100 ± 0*	>3.0 ± 0.01*
25	100 ± 0*	>3.0 ± 0.02*
40	98.3 ± 0.5	1.8 ± 0.1

*p-value <0.05 compared to 40°C

Four-component wash solution consisted of a mixture of 0.013 g/ml L-ascorbic acid, 0.112 g/l citric acid, 0.01 g/ml ethylenediaminetetraacetic acid, and 0.031 g/ml sodium lauryl ether sulfate, and adjusted to pH 3.0.

Growth of *E. coli* was measured using a plate counting assay.

g/l: gram per liter; g/ml: gram per milliliter; SD: standard deviation

decreased *E. coli* O157:H7 survival. Utilization of 10% (v/v) CA solution at pH 9.5 results in significant membrane damage and viability loss of *E. coli* and *Klebsiella pneumoniae* amounting to 4.6 $\log_{10}$ CFU/ml reduction in both microorganisms (Burel *et al*, 2021). The efficacy of CA at high pH could possibly due to its zeta potential which makes the bacterial cell surface more negative and thereby increasing its sensitivity to CA that leads to cell membrane instability (Burel *et al*, 2021).

Although citric acid solution alone is an effective antimicrobial agent, it is prudent to apply it in combination with other GRAS compounds to minimize bacterial adaptation to acid stress and to avoid the possibility of development of resistance to the organic acid, the basis of HET (Chapman and Ross, 2009). Three GRAS compounds were chosen in the current study, namely, ascorbic acid, EDTA and SLS. Ascorbic acid is an antioxidant which scavenges reactive free radicals and other reactive oxygen species (ROS) formed in cell metabolism (Ames *et al*, 1993). EDTA is a metal chelator and possesses weak broad-spectrum antimicrobial activity towards planktonic and biofilm cultures (Reardon *et al*, 1991; Yakandawala *et al*, 2007). SLS is a surfactant of biofilms and surface-active amphiphile, the latter property decreases surface tension of water (Florence and Attwood, 2006), thereby allowing efficient contact with microbes. We successfully implemented the HET approach by combining these four GRAS compounds into a single wash solution employing, which achieved approximately 2 $\log_{10}$ or greater reduction in *E. coli* O157:H7 growth following exposure for 5 minutes at 25°C and 4°C. The slight reduction in bacterial growth inhibition at 40°C could be attributed to an increase in the rate of ascorbic acid oxidation, which reduces its antioxidant property (Rahmawati and Bundjali, 2012).

Kang and Song (2015) showed that a combination of 1.0% (v/v) CA with various concentrations (0.05, 0.1, 0.2, and 0.3%) of Tween 20 exhibit a decrease in growth on perilla leaves of aerobic mesophilic bacteria, such as *E. coli* and *Salmonella typhimurium,* by 2.61 $\log_{10}$ CFU/ml. Sangcharoen *et al* (2017) reported that a solution of ascorbic acid and EDTA enhances antimicrobial potency. Field *et al* (2017) demonstrated that a combination of 500 ppm of nisin, a polycyclic antibacterial peptide produced by *Lactococcus lactis* commonly used as a food preservative, 2,000 ppm of ascorbic acid and 250 ppm of EDTA results in 3.41 $\log_{10}$ reduction of *Salmonella enteritidis* ATCC 13076 growth. Furthermore, 10 μM nisin combined with 0.25-2.0% (w/v) EDTA and 125 μg/ml essential oil trans-cinnamaldehyde controls growth of *E. coli* in swine, and *in vitro* reduced bacterial growth by 90% following a 3-hour incubation.

We showed that household wash solutions, distilled water, 0.04% (v/v)

hydrogen peroxide, 20.0% (w/v) sodium chloride, and 2.50% (v/v) vinegar (containing 4.5% (v/v) acetic acid, *ie* 0.11% (v/v) acetic acid) were markedly poor bactericidal wash solutions. Jensen *et al* (2015) demonstrated that washing of *E. coli* O157:H7-inoculated lettuce leaves result in transfer of only 90-99% of bacteria to wash water. Similarly, Uhlig *et al* (2017) reported that washing lettuce in distilled or tap water do not significantly remove bacteria, such as *E. coli* and other Enterobacteriaceae, even after the fifth washing. These results are expected because water has no bactericidal property and removal of microbes is dependent on a mechanical effect. As regards hydrogen peroxide, there is a possibility that the low concentration used activated *topA* P1 promoter expression mediated by DNA-binding protein Fis, a component of *E. coli* response to oxidative stress (Weinstein-Fischer *et al*, 2000), but this conjecture needs to be shown.

Doyle and Glass (2010) suggested that higher salt levels significantly increase time of growth by one generation in pathogens such *E. coli*, *Clostridium botulinum* and *Salmonella* spp. At higher salt levels, these microbes adapt to a hyperosmotic environment by accumulating potassium, amino acids and/or sugars to counteract the osmotic pressure, and also increase activity of sodium efflux systems (Christian, 2000; Lado and Yousef, 2007) as well as elevate production of stress proteins (Duché *et al*, 2002).

Park *et al* (2016) reported treating a laver for 7 days with 5, 10 and 15% dilution of vinegar (containing 6% (v/v) acetic acid, *ie* 0.3, 0.6 and 0.9% (v/v) acetic acid) reduces *E coli* counts by 3.4, 2.5 and 2.0 $\log_{10}$ folds respectively. However, Gomez-Aldapa *et al* (2018) noted that diluted vinegar containing 0, 0.05 or 0.5% (v/v) acetic acid showed no significant effects on *E. coli* survival. Thus, antibacterial activity of vinegar is related to both acetic acid content and contact time (Bakir *et al*, 2017).

In summary, the study demonstrates a 0.112 mg/ml citric acid solution was more effective in reducing *E. coli* O157:H7 numbers compared to a commercial food disinfectant wash solution. However, by applying a hurdle effect technology (HET), whereby citric acid was combined with other generally accepted as safe (GRAS) compounds, namely, L-ascorbic acid, ethylenediaminetetraacetic acid and sodium lauryl ether sulfate, enhanced the bactericidal activity of citric acid solution alone. Standard household disinfectant wash solutions (distilled water, low concentration of hydrogen peroxide, high salt and diluted vinegar) were marked less superior to the four-GRAS component mixture. However, the total cost of the components is higher than the commercial wash solution (data not shown). This four-component wash solution should be further tested against other foodborne pathogens and acid resistant species/strains.

Fresh food products should be evaluated for the presence of pathogenic microbes and for efficacy of the four-component wash solution under non-laboratory settings. Probabilistic modelling or response surface methodology could be implemented in designing less costly GRAS components. Importantly, the allowable concentration ranges as approved by Food and Drug Administration (FDA) guidelines should be taken into consideration in formulating mixtures of other GRAS compounds as disinfectant wash solutions for food and food products.

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CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

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