Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

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Ciência e Tecnologia de Alimentos, vol. 30, núm. 4, octubre-diciembre, 2010, pp. 1083-1089
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

Original

Hygienic-sanitary quality of vegetables and evaluation of treatments for the elimination of indigenous *E. coli* and *E. coli* O157:H7 from the surface of leaves of lettuce (*Lactuca sativa* L.)

Qualidade higiênico-sanitária de hortaliças e avaliação de tratamentos para eliminação de E. coli indígena e E. coli O157:H7 na superfície de folhas de alface (Lactuca sativa L.)

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Abstract

The purpose of this study is to evaluate the hygienic-sanitary quality of vegetables and irrigation water and assess the effectiveness of lemon juice and vinegar in reducing *E. coli* strains inoculated on lettuce. One hundred and forty samples of vegetables and 45 samples of irrigation water were investigated for thermotolerant coliforms and *Salmonella* spp. In order to verify the effectiveness of natural household sanitizers in reducing *E. coli* in inoculated lettuce, four treatment solutions were tested: fresh lemon juice, alcohol vinegar, lemon juice-vinegar mixture, and lemon juice-vinegar-water mixture. The microbiological analysis revealed high rates of contamination by thermotolerant coliforms and identified the presence of *E. coli* in 32% of the tested vegetable samples and 56% of the water samples. While no significant statistical difference ($p < 0, 05$) was identified in the tested solutions, the treatment with a combination of lemon juice and vinegar resulted in the highest Decimal Reductions (DR) of *E. coli* O157: H7 while the treatment with vinegar alone was the most effective against the indigenous *E. coli* strain.
Keywords: *Escherichia coli; natural household sanitizers; vegetables; irrigation water.*

Resumo

O objetivo desse estudo foi avaliar a qualidade higiênico-sanitária de hortaliças e água de irrigação e a eficácia do uso de suco de limão e vinagre na redução de cepas de *E. coli* inoculadas em alface. Cento e quarenta amostras de hortaliças e 45 amostras da água de irrigação foram coletadas e investigadas para a presença de coliformes termotolerantes e *Salmonella* spp. Para verificar a eficácia de sanitizantes naturais de uso doméstico na redução de *E. coli* na alface inoculada, quatro tratamentos foram testados: suco de limão fresco, vinagre de álcool, mistura suco de limão-vinagre e mistura suco de limão-vinagre-água. A análise microbiológica revelou altas taxas de contaminação por coliformes termotolerantes e identificou a presença de *E. coli* em 32% das amostras das hortaliças investigadas e em 56% das amostras de água. Embora nenhuma diferença estatística significativa ($p < 0, 05$) tenha sido identificada entre as soluções testadas, o tratamento com a combinação de suco de limão e vinagre forneceu a mais alta Redução Decimal (RD) para *E. coli* O157: H7, enquanto o uso de vinagre apenas foi o tratamento mais efetivo contra a cepa *E. coli* indígena.
Palavras-chave: *Escherichia coli; sanitizante natural de uso doméstico; hortaliças; água de irrigação.*

1 Introduction

Whole, cut, and minimally processed fresh fruits and vegetables and juices are recognized as nutritional foods. In Brazil the widespread habit of consuming raw vegetables allows for the transmission of diseases caused by bacteria, parasites, and viruses. Vegetables are cultivated throughout the year, sometimes in soil beds fertilized with animal manure. Plant roots are in constant contact with the soil and are often irrigated with polluted water. Studies have demonstrated that pathogens present in contaminated soil may remain viable for up to 2 months or more, especially in moist and shaded areas. In recent years, the frequency of outbreaks of foodborne illnesses associated with the consumption of fresh vegetables and fruit has increased, partly because of an increased demand for fresh produce (CHANG; CHEN, 2003; LYNCH; TAUXE; HEDBERG, 2009). Salads containing raw vegetables have been identified as vehicles of traveler's diarrhea, an illness sometimes experienced by visitors to developing countries. Enterotoxigenic *Escherichia coli* is the most common cause of this illness. Enterohemorrhagic *E. coli*, specifically serotype O157: H7, has been implicated as the causative agent in outbreaks of gastroenteritis in the USA, Japan, and other countries (BEUCHAT, 1996).

Chlorinated water is generally used to remove bacterial pathogens from vegetables. However, studies have shown that washing with chlorinated water has a limited bactericidal effect

Recebido para publicação em 19/2/2009
Aceito para publicação em 31/10/2009 (004075)
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(LIN et al., 2000). Other sanitizers studied for their effectiveness in removing pathogens from fresh fruit and vegetables include trisodium phosphate (ZHUANG; BEUCHAT, 1996, WEBER; OBRIEN; BENDER, 2004; MOLINOS et al., 2005), organic acids (PIROVANI et al., 1998; RHEE et al., 2003; BJORNSDOTTIR; BREIDT; McFEETERS, 2006), hydrogen peroxide (BRENNAN; PORT; GORMLEY, 2000), and ozone (KOSEKI et al., 2001; ACHEN; YOUSEF, 2001; BELTRAN et al., 2005). Although various sanitizers have been examined for their effectiveness, most treatments have been shown to have minimal effects, resulting in less than a 2 log cfu.g^{-1} (cm^2) reduction in bacterial numbers, and most would not be suitable products for use at the household level (BEUCHAT et al., 1998). Furthermore, consumers are increasingly avoiding consuming foods treated with preservatives of chemical origin, and therefore natural alternatives are needed to achieve a high-degree of safety with respect to foodborne pathogenic microorganisms (RAUHA et al., 2000). Studies into the lethal effects of organic acids namely ascorbic, citric, lactic, malic, propionic, succinic, and tartaric acids, which are naturally found in a variety of fruits and fermented foods, have been carried out by several researchers (BUCHANAN; EDELSON, 1999; ESCUDERO et al., 1999; BRENNAN; PORT; GORMLEY, 2000; RHEE et al., 2003; BJORNSDOTTIR; BREIDT; McFEETERS, 2006), and the antimicrobial activity of these acids is well documented. Vinegar and lemon juice, natural sources of acetic and citric acids, respectively, are used as flavouring and for acidifying liquids for vegetable salads and may be considered as safe alternative disinfectants to reduce pathogens.

The purpose of this study is to evaluate the hygienic sanitary quality of fresh vegetables and irrigation water and to determine the sanitizing effect of vinegar, lemon juice, vinegar-lemon juice mixture, and vinegar-lemon juice-water mixture on *Escherichia coli* strains inoculated on lettuce.

2 Materials and methods

2.1 Microbial evaluation of vegetables and irrigation water

Study area and products sampled

In the course of this study, five vegetable growing areas were investigated between July and December of 2005. The areas investigated in this work are located in three neighborhoods in the Cabula-Beiru region in Salvador, Bahia. The crops in those areas have been produced for 8 to more than 30 years, and the total area used for agriculture ranges from 5.798 to 35.176 m^2. They grow mainly vegetables, producing about 130 to 1,150 heads per day destined for open markets and local stores in the city of Salvador. One hundred and forty samples of vegetables ranging from lettuce (*Lactuca sativa*), cilantro (*Coriadrum sativum*), kale cabbage (*Brassica oleracea*), and mint (*Mentha villosa*) plus 45 samples of irrigation water were taken.

Sampling procedures

The vegetables were randomly selected from the plants harvested, the edible portions (leaves) were separated using knives previously sanitized with hypochlorite, then placed in clean plastic sealable bags, and transported to the laboratory in insulated coolers. For testing the irrigation water, samples were collected in sterile glasses from: water from wells, ponds, brooks, and public water system (from a pump used for irrigation) (MESSER; MIDURA; PEELER, 1992).

Laboratory procedures

Vegetables: In the laboratory a 25 g random subsample of the vegetable matter was homogenized in a stomacher with 225 mL 0.1% Buffer Peptone Water (BPW), and decimal dilutions were prepared (MESSER; MIDURA; PEELER, 1992).

Thermotolerant coliforms were investigated by the Most Probable Number (MPN) technique, using series of three tubes and Hoskins table, according to Hitchins, Hartman and Todd (1992). The tubes showing gas production in EC broth were streaked on Eosin Methylene Blue (EMB) agar to investigate the presence of *E. coli*. Colonies appearing bluish with greenish metallic sheen on EMB plates were confirmed by biochemical tests of IMViC.

The presence of *Salmonella* spp. was investigated according to Flowers et al. (1992) with the pre enrichment step in 0.1% BPW, enrichment in Tetrathionate and Rappaport broth (42 °C/24 hours), and isolation on Hektoen Enteric, *Salmonella-Shigella* and Brilliant Green agar (35 °C/24 hours).

Irrigation water: Total and thermotolerant coliforms were investigated using the MPN technique according to Clesceri, Greenberg and Eaton (1988). Five tubes containing 10 mL of 2X Lauryl Sulfate Tryptose broth (LST) and 10 mL of the sample (first dilution) were used for raw water. For the other dilutions, 1 mL of the sample was inoculated in 9 mL of 1X LST broth. Potable water was investigated by inoculating a volume of 100 mL of the sample in 10 tubes containing 10 mL of the 2X LST. Total coliforms were confirmed in 2% lactose bile brilliant green broth. Thermotolerant coliforms were confirmed in EC broth at 44.5 °C/24 hours. The tubes showing gas production in EC broth were streaked on Eosin Methylene Blue (EMB) agar to investigate the presence of *E. coli*. Colonies appearing bluish with greenish metallic sheen on EMB plates were confirmed by biochemical tests of IMViC.

2.2 Antimicrobial effects of vinegar and lemon juice against indigenous E. coli and E. coli O157: H7 inoculated on lettuce

Lettuce: Fresh iceberg lettuce (*Lactuca sativa*) was purchased from a local supermarket in Salvador - BA, Brazil. After aseptically removing the outer leaves and core, the inner leaves were washed with cold tap water at 21 °C and cut into 3 × 3 cm pieces using a sterile knife. These shredded lettuce pieces were treated by UV light (30 W, 50 cm irradiation distance) in a class II biosafety cabinet (TROX TECHNICK) for 30 minutes (15 minutes for each side) to reduce the native microflora (SEGUN; KARAPINAR, 2004).

Preparation of inocula: *E. coli* isolated from lettuce in the first step of this study and *E. coli* O157: H7 (calf feces isolated) cells were activated separately by two successive 24 hour transfers in Brain Heart Infusion broth (BHI) at 37 °C with shaking at 120 rpm to achieve viable cell population of 10^{10} cfu.mL^{-1}. The cell suspensions were transferred to sterile eppendorf tubes,

and the inoculum levels were confirmed by surface plating duplicate samples on TSA. The plates were incubated at 37 °C for 24 hours before colony counts were obtained. This experiment was repeated three times in duplicate (BLANCO et al., 1992; SINGH et al., 2002; SEGUN; KARAPINAR, 2004, 2005). Cell suspensions were diluted in an appropriate amount of water to give a cell number of 10^8 cfu.mL^{-1} and used immediately for sample inoculation.

Preparation of treatment solutions: Four treatment solutions were tested: fresh lemon juice, alcohol vinegar, lemon juice-vinegar mixture, and lemon juice-vinegar-water mixture. Lemons (TAITI variety) were purchased from a local supermarket and washed with tap water. After cutting the lemons with a sterile knife, a sterile domestic citrus fruit squeezer was used in experiments to squeeze the lemons. In all experiments, pasteurized alcohol vinegar (MINHOT brand) was used. The lemon juice-vinegar mixture was prepared by combining lemon juice with vinegar at the ratio of 1:1, and the diluted mixture was prepared by combining lemon juice with vinegar and water at the ratio of 1:1:1 under aseptic conditions. The acidity of the treatment solutions was determined by using a volumetric method (v/v) and expressed as acetic acid in vinegar, lemon juice-vinegar mixture, and lemon juice-vinegar-water mixture (ASSOCIATION..., 2000a) or citric acid in lemon juice (ASSOCIATION..., 2000b).

Inoculation of lettuce: The dip inoculation method was used to inoculate the lettuce leaves. Cell suspensions of indigenous *E. coli* isolated from the lettuce and *E. coli* O157: H7 were prepared by transferring 2 mL of the activated cultures into 200 mL of 0.1% BPW, so that the final cell number in the suspension was approximately 10^8 cfu.mL^{-1}. Sixty grams of shredded lettuce leaves were dipped into 200 mL of inoculum prepared as described earlier and then shaken gently using an environmental incubator shaker (INNOVA, New Brunswick Scientific) at 120 rpm for 1 minute at room temperature (21 °C) to ensure an even distribution of organisms. During shaking, the lettuce leaves were completely submerged in the inoculum. To allow the attachment of *E. coli*, the inoculated lettuce leaves were air-dried under a class II biosafety cabinet for 1 hour at 21 °C before undergoing treatment (SINGH et al., 2002).

Treatment of lettuce: Portions of 10 g of inoculated shredded lettuces leaves were submerged in 50 mL of each treatment solution (vinegar, lemon juice, vinegar-lemon juice, and vinegar-lemon juice-water) and in the control (BPW, pH 7.2)) at room temperature (22 °C) for 15 minutes. Immediately after decanting the treatment solution, *E. coli* was counted (SEGUN; KARAPINAR, 2004, 2005; SINGH et al., 2002).

Procedure for enumerating indigenous *E. coli and E. coli* O157: H7*:* To count *E. coli* O157:H7, the shredded lettuce leaves (10 g) were transferred into sterile Stomaching bags combined with 90 mL of BPW and then pummeled in a Bag Mixer (NOVA ETICA, Brazil) at medium speed for 2 minutes. The resulting slurry was serially diluted and surface plated (0.1 mL) in duplicate on Sorbitol MacConkey agar (CT-SMAC) (HIMEDIA) supplemented with cefixime-tellurite (0.05, 2.5 mg.L^{-1}). The plates were incubated at 37 °C for 24 hours before the colonies were counted. Non-sorbitol-fermenting colonies on CT-SMAC, were confirmed for the presence of shiga toxin using VERO cells (SALVADORI; DELLA COLLETA; TOMOMASA, 1997) and the Bacto *E. coli* O157: H7 antiserum assay (BLANCO et al., 1992, 1996). Confirmed populations are expressed as cfu.g^{-1} of lettuce.

To count indigenous *E. coli*, the resulting slurry was serially diluted and surface plated (0.1 mL) in duplicate on Levine's Eosin-Methylene blue agar (L-EMB). The plates were incubated at 37 °C for 24 hours before the colonies were counted. The isolates were confirmed using biochemical reactions of IMViC (HITCHINS; HARTMAN; TODD, 1992).

2.3 Statistical analysis

To investigate the effectiveness of lemon juice, vinegar-lemon juice mixture, and vinegar-lemon juice-water in the elimination of *E. coli* two replicate experiments were conducted, with three samples evaluated per replicate and all counts of bacteria (cfu.g^{-1}) recovered from the lettuce leaves were transformed to logarithms before computing means and standard deviations. The Decimal Reduction (DR) in the population of the bacteria was calculated by the difference between the counts obtained in the control and each treatment. The data were subjected to the Statistical Analysis System for analysis of variance and Duncan's multiple range test (SPSS 2.3 for Windows pocket program) to determine whether significant differences ($p < 0.05$) in populations of *E. coli* existed among mean log values.

3 Results and discussion

3.1 Indicator of microorganisms prevalence in vegetables and irrigation water

Sixty percent of the growing areas presented insalubrious conditions with the presence of trash, discarded objects, rodents, and pets. These conditions represent risks to crop cultivation and propitiate the presence of pathogenic bacteria in the fresh vegetables. The presence of a sewer was observed in two (40%) areas investigated and none of them had sanitary installations for workers. Irrigation was performed with water mainly from wells, ponds, and brooks; only one area used the public water system. All areas made use of bovine or poultry manure without pre-treatment to destroy pathogens. Workers had no health care, and only one of them had visited a physician in the last six months.

The technique of composting manure has not been widely employed in Brazil yet. According to Ingham et al. (2004), bovine manure is a well-known source of food-borne pathogenic bacteria and using it without prior treatment to destroy pathogens increases the likelihood of contaminating vegetables grown in manure-fertilized soils. Composting is an accepted manure pathogen reduction treatment, and compost-generated heat is believed to eliminate pathogenic bacteria (INGHAM et al., 2004).

The results obtained in the investigation of Most Probable Number (MPN) of thermotolerant coliforms in vegetables and irrigation water samples are presented in Table 1. The results show high levels of contamination mainly in lettuce and cilantro cultivated in patches 2 and 3. In general, with regard to lettuce

Table 1. Most probable number of thermotolerant coliforms in vegetables and irrigation water.

Vegetables	Patch 2	Patch 3	Patch 5	Patch 6	Patch 9
Lettuce	110 to 1500	15 to 1500	<0.3 to 1500	<0.3 to 1500	<0.3 to 430
Cilantro	110 to 1500	2.3 to 1500	4.0 to 1100	<0.3 to 1100	<0.3 to 1100
Kale cabbage	<0.3 to 1100	<0.3 to 4.3	<0.3 to 110	<0.3 to 110	<0.3 to 110
Mint	<0.3 to 1500	<0.3 to 1500	<0.3 to 1500	<0.3 to 1500	<0.3 to 1100
Irrigation water	30 to > 1600	240 to >1600	< 2.0 to 1600	240 to >1600	>1600

and cilantro, all patches showed samples with high levels of contamination.

Cilantro was the vegetable with the highest number of positive samples for the microorganism group and *E. coli*, followed by lettuce and mint (Table 2). There were fewer positive samples for kale cabbage, probably because it has a stem which keeps the leaves out of direct contact with the soil. *Salmonella* was not detected in the samples analyzed in this study.

Similar results were found by Ribeiro et al. (2005) and Takayanagui et al. (2001) in São Luis - MA, Brazil and Ribeirão Preto - SP, Brazil, respectively. The Brazilian standards are fixed at 10^2 MPN.g^{-1} only for ready to eat vegetables (BRASIL, 2001). Considering the fact that these vegetables are eaten raw and that pre-sanitization is not carried out, the risk of foodborne disease is present. Furthermore, sanitization procedures would be ineffective because of the high population of microorganisms present in raw vegetables.

E. coli was isolated from all vegetables investigated and from the samples of the cilantro presented the highest frequency (Table 2). Patches 2, 3, and 5 showed the highest frequency of contaminated vegetables (38, 46 and 39%, respectively) (Table 3). In general, *E. coli* was isolated in 32% of the samples investigated, which confirms the recent fecal contamination of the vegetables.

The persistence of fecal bacteria in the soil has been reported elsewhere. Jones (1999) described *E. coli* that survived for at least 60 days in soil at 25 °C and for at least 100 days at 4 °C. Bolton et al. (1999) detected *E. coli* O157: H7 in soil 99 days after a fecal suspension containing this organism was applied to grassland. In the study by Ingham et al. (2004), when no manure was applied, bird and/ or mammal recontamination was the cause of the apparent persistence of indigenous *E. coli* in manure-fertilized soils. According to the authors, the results of the manure-fertilized soil analyses suggested that the application of non-composted bovine manure sufficiently in advance of vegetable harvesting would present little additional risk beyond that associated with nonfertilized soil.

In the present study, 89% of the irrigation water samples were positive for coliform (MPN ranging from <2.2 to 1600); 87% presented thermotolerant coliforms (MPN ranging from <2.0 to > 1600) (Table 1). All of the investigated patches had contaminated samples. *E. coli* was isolated from 56% of the samples and the patch 3 presented the highest number of samples containing the microorganism (Table 4).

Innumerous studies have shown that irrigation water is the main cause of contamination of vegetables in agriculture

Table 2. Frequency of thermotolerant coliforms and *Escherichia coli* in vegetables and irrigation water.

Vegetables	Samples (n)	Thermotolerant coliforms/*E. coli*	
		Positive samples (n)	Positive samples (%)
Lettuce	35	31/12	89/34
Cilantro	35	33/15	94/43
Kale cabbage	35	22/6	63/17
Mint	35	29/12	83/34
Irrigation water	45	39/25	87/56

Table 3. Frequency of thermotolerant coliforms and *Escherichia coli* in vegetables according to the patches investigated.

Patches	Samples (n)	Thermotolerant coliforms/*E. coli*	
		Positive samples (n)	Positive samples (%)
Patch 2	24	22/9	92/38
Patch 3	24	21/11	88/46
Patch 5	36	30/14	83/39
Patch 6	20	15/4	75/20
Patch 9	36	27/7	75/19
Total	140	115/45	82/32

Table 4. Frequency of thermotolerant coliforms and *Escherichia coli* in irrigation water according to the patches investigated.

Patches	Samples (n)	Thermotolerant coliforms/*E. coli*	
		Positive samples (n)	Positive samples (%)
Patch 2	10	10/7	100/70
Patch 3	10	10/9	100/90
Patch 5	10	9/2	90/40
Patch 6	10	5/5	50/50
Patch 9	5	5/2	100/40
Total	45	39/25	87/56

(TAKAYANAGUI et al., 2001; SILVA; ANDRADE; STAMFORD, 2005). The Brazilian microbiological standard for irrigation water is 200 thermotolerant coliforms.mL^{-1} (BRASIL, 2005). The results of this work show that 68.9% of the samples exceeded the established limit, and areas 3 and 9 presented the highest number of samples with MPN values above 200.mL^{-1}.

According to Del Rosario and Beuchat (1995), the growth of *E. coli* O157:H7 in apple cider and raw produce has been demonstrated. The pathogen was the likely cause of 14 cases of hemolytic uremic syndrome attributed to the consumption of apple juice. It can also grow on shredded lettuce and sliced cucumber at 12 and 21 °C.

Other agricultural factors, such as the application-to-planting interval, harvest hygiene, and postharvest washing treatments are also critically important in preventing contamination of vegetables with pathogenic bacteria.

3.2 Antimicrobial effects of vinegar and lemon juice against indigenous E. coli and E. coli O157: H7 inoculated on lettuce

The efficacy of treatments with lemon juice, vinegar, lemon juice-vinegar mixture, and diluted lemon juice-vinegar mixture against *E. coli* O157:H7 and indigenous *E. coli* inoculated lettuce is shown in Tables 5 and 6, respectively.

As a control, the populations of *E. coli* O157:H7 and indigenous *E. coli* were counted in inoculated lettuce samples without vinegar or lemon juice treatments.

When lettuce leaves were treated with the lemon juice-vinegar mixture, the observed reduction of *E. coli* O157:H7 ranged from 2.52 to 4.19 log cfu.g^{-1}, with a mean of 3.25. However, there was no statistical difference between the lettuce samples treated only with vinegar and lemon juice or with the lemon juice-vinegar mixture ($p > 0.05$) (Table 5). The treatment using the diluted mixture of lemon juice-vinegar showed the smallest reduction (1.05 log reduction) in the population of *E. coli,* and a statistical difference was observed ($p < 0.05$).

Segun and Karapinar (2004) found similar results on carrots inoculated with *Salmonella* Typhimurium. According to the authors, the initial populations of *S.* Typhimurium were reduced to 4.11 log cfu.g^{-1} when dipping carrots in a mixture of lemon juice and vinegar (1:1), and the treatment with lemon juice was more effective in eliminating viable *S.* Typhimurium cells than did the treatment with vinegar. These results are corroborated in the present work.

Indigenous *E. coli* isolated from lettuce leaves (first step of this work) and inoculated on pieces of lettuce leaves showed higher resistance to the treatments than did *E. coli* O157:H7. Unlike the results demonstrated for *E. coli* O157:H7 inoculated lettuce, vinegar alone was the best treatment against indigenous *E. coli*, but a statistical difference was not observed ($p > 0.05$) when compared to lemon juice and vinegar-lemon juice mixture.

Table 5. Effectiveness of vinegar and lemon juice in inactivating *E. coli* O157:H7 inoculated on lettuce.

Treatment solutions	Acidity	Inoculated population (log cfu.g^{-1})	Population recovered (log cfu.g^{-1})	Log reduction (log cfu.g^{-1})
Lemon juice	3.66 (citric acid)	6.15 ± 0.15	3.40 ± 0.13	2.75
		6.23 ± 0.20	3.40 ± 0.11	2.83
		6.08 ± 0.12	5.38 ± 0.62	0.70
				Mean = 2.09^{a*} ± 1.2
Vinegar	4.20 (acetic acid)	6.15 ± 1.10	3.90 ± 0.67	2.25
		6.23 ± 0.13	2.70 ± 0.54	3.53
		6.08 ± 0.11	3.30 ± 0.56	2.78
				Mean = 2.85^{a*} ± 0.64
Vinegar + lemon juice (1:1)	8.04 (acetic acid)	6.15 ± 0.75	3.05 ± 0.13	3.05
		6.23 ± 0.73	2.04 ± 0.17	4.19
		6.08 ± 0.64	3.56 ± 0.12	2.52
				Mean = 3.25^{a*} ± 0.84
Vinegar + lemon juice + water (1: 1: 1)	5.28 (acetic acid)	6.26 ± 0.11	4.34 ± 0.14	1.92
		6.34 ± 0.05	5.87 ± 0.11	0.47
		6.53 ± 0.12	5.76 ± 0.17	0.77
				Mean = 1.05^{A*} ± 0.76

* Values sharing a common letter are not significantly different ($p > 0.05$); ± Standard deviation.

Table 6. Effectiveness of vinegar and lemon juice in inactivating indigenous *E. coli* inoculated on lettuce.

Treatment solutions	Acidity	Inoculated population (log cfu.g^{-1})	Population recovered (log cfu.g^{-1})	Log reduction (log cfu.g^{-1})
Lemon juice	3.66 (citric acid)	6.30 ± 0.19	5.46 ± 0.16	0.84
		6.48 ± 0.23	5.86 ± 0.13	0.62
		6.67 ± 0.32	5.67 ± 0.14	1.00
				Mean = 0.82^{b*} ± 0.19
Vinegar	4.20 (acetic acid)	6.30 ± 0.21	5.18 ± 0.16	1.12
		6.48 ± 0.18	5.04 ± 0.41	1.44
		6.67 ± 0.15	4.70 ± 0.35	1.97
				Mean = 1.51^{b*} ± 0.42
Vinegar + lemon juice (1:1)	8.04 (acetic acid)	6.30 ± 0.13	5.08 ± 0.17	1.22
		6.48 ± 0.16	5.20 ± 0.15	1.28
		6.67 ± 0.25	5.43 ± 0.31	1.24
				Mean = 1.25^{b*} ± 0.30
Vinegar + lemon juice + water (1: 1: 1)	5.28 (acetic acid)	6.30 ± 0.37	5.46 ± 0.14	0.84
		6.48 ± 0.11	5.79 ± 0.23	0.69
		6.67 ± 012	5.41 ± 0.30	1.26
				Mean = 0.93^{b*} ± 0.29

* Values sharing a common letter are not significantly different ($p > 0.05$); ± Standard deviation.

According to Buchanan and Edelson (1999), the acid resistance of stationary-phase EHEC cells varies substantially among strains and depends on the acidulant present and on whether the pH-dependent acid resistance system has been induced .The antimicrobial activity of weak organic acids (citric, malic, lactic, or acetic acid), according to the authors, increased the rate of inactivation making it possible to demonstrate increased stationary-phase acid resistance when cells were initially grown in acidogenic TSB + G. One possibility to rationalize the differences in acid resistance among the strains is that EHEC becomes "animal or commodity adapted". The decreased antimicrobial activity of malic and citric acids may also reflect the lower concentrations of undissociated acid if compared to lactic acid. However, this would not account for lactic acid being more effective than acetic acid. The calculated concentration of undissociated acetic acid is almost twice as high as that of lactic acid.

4 Conclusions

Overall. the results of this study revealed that the vegetable growing areas investigated were in poor conditions and had a high number of samples of vegetables and great amount of irrigation water contaminated with thermotolerant coliforms and *E. coli*. Interventions can and should be made whenever and wherever possible to eliminate or control the growth of pathogenic bacteria on fresh produce. The use of natural household sanitizers in the elimination of *E. coli* can be considered potential antimicrobial agents in preventing foodborne outbreaks related to fresh produce. The effectiveness of the lemon juice and vinegar against *E. coli* on shredded lettuce was affected by the type of strain present in the vegetable. *E. coli* O157:H7 was more sensitive to vinegar, lemon juice, and lemon juice-vinegar mixture than did indigenous *E. coli*. Despite there being no statistical difference between the lettuce samples treated with vinegar, lemon juice, and lemon juice-vinegar mixture ($p > 0.05$), the population of *E. coli* O157:H7 decreased in the samples treated. Therefore, the use of these 2 sanitizers, alone or in combination, can be considered an efficient decontamination technique for leafy vegetables.

Acknowledgements

We would like to thank "Laboratorio Gonçalo Muniz (LACEN)" and "Biotecnologia e Ecologia de Microrganismos" laboratory staff, who collaborated with us in this study. We would like to acknowledge Dr. Tomomasa Yano for helping with the analysis for detecting the presence of the shiga toxin.

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