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Evaluation of antibacterial efficiency of commercial acetic fermented in lettuce salad (*Lactuca sativa*) marketed in Duque de Caxias city, Rio de Janeiro

Avaliação da eficiência antibacteriana de fermentados acéticos comerciais em saladas de alface (*Lactuca sativa*) comercializadas na cidade de Duque de Caxias, Rio de Janeiro

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ABSTRACT

Introduction: Contamination of raw consumed foods such as lettuce salads is predictable by telluric origin and improper handling in commercial establishments constituting a possible risk to Public Health. The use of acetic fermented as antibacterial could be a skillful mechanism to improve the quality and safety of this food. **Objective:** To evaluate the microbiological quality of lettuce (*Lactuca sativa*) salads marketed in the city of Duque de Caxias, RJ and the effectiveness of acetic acid lecithin as a decontaminant **Method:** Lettuce salad samples were collected at four restaurants and analyzed before and after decontamination with acetic fermented in volatile acidity (4, 5 and 6 in g. 100 mL^{-1}) and concentrations of 10% and 50% the count of mesophilic aerobic bacteria, total and thermotolerant coliforms and the presence of *Salmonella* spp. **Results:** The analyses revealed poor quality of the samples in three restaurants (A, B, C) where, although the presence of Salmonella spp. was not observed, there were high counts of mesophilic aerobic bacteria and total and thermotolerant coliforms. In accordance with legal regulations, in two establishments, the samples would be disapprove by the excess of thermotolerant coliforms. With the use of acetic fermentation in the decontamination, bacterial counts were reduced, allowing all the samples to present satisfactory conditions for human consumption. There was no significant difference ($p < 0.05$) between the treatments with different concentrations and acidity of acetic fermented in relation to the untreated samples. **Conclusions:** Acetic fermented can be a safe and practical alternative to improve the hygienic-sanitary quality of lettuce in raw salads, ensuring food safety and reducing risks to human health.

KEYWORDS: *Lactuca sativa*; Acetic Acid; Microbiological Analysis; Decontamination; Food Safety

RESUMO

Introdução: A contaminação de alimentos consumidos crus, como salada de alface, é previsível pela origem telúrica e pela manipulação indevida nos estabelecimentos comerciais constituindo um possível risco à saúde pública. A utilização de fermentado acético como antibacteriano poderia ser um mecanismo hábil para melhorar a qualidade e a segurança desse alimento. **Objetivo:** Avaliar a qualidade microbiológica das saladas de alface (*Lactucasativa*) comercializadas na cidade de Duque de Caxias, RJ, e a efetividade do fermentado acético de maçã como descontaminante. **Método:** Foram coletadas amostras de saladas de alface em quatro restaurantes, que foram analisadas antes e após a descontaminação com fermentados acéticos em acidezes voláteis (4, 5 e 6 em g. 100 mL^{-1}) e concentrações de 10% e 50% quanto à contagem de bactérias aeróbicas mesófilas, coliformes totais e termotolerantes e quanto à presença de *Salmonella* spp. **Resultados:** As análises revelaram má qualidade das amostras em três restaurantes (A, B,

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C) nos quais, embora não tenha sido constatada a presença de *Salmonella* spp., verificou-se contagens elevadas de bactérias aeróbias mesófilas e de coliformes totais e termotolerantes. De acordo com as normas legais, em dois estabelecimentos as amostras seriam reprovadas pelo excesso de coliformes termotolerantes. Com o uso do fermentado acético na descontaminação, houve redução das contagens bacterianas, permitindo que todas as amostras apresentassem condições satisfatórias para consumo humano. Não houve diferença significativa ($p < 0,05$) entre os tratamentos com diferentes concentrações e acidezes de fermentado acético em relação as amostras não tratadas. **Conclusões:** O fermentado acético pode ser uma alternativa segura e prática para melhorar a qualidade higiênico-sanitária da alface em saladas cruas, garantir a segurança alimentar e reduzir os riscos à saúde humana.

PALAVRAS-CHAVE: *Lactuca sativa*; Ácido Acético; Análise Microbiológica; Descontaminação; Segurança Alimentar

INTRODUCTION

Consumption of green leaves like lettuce (*Lactuca sativa*) has been increasing because of their nutritional benefits and convenience, since they are considered ready-to-eat (RTE) food[1,2].

Behrens et al.[3] observed that these types of food, although convenient because they save time, also raise suspicions about integrity and safety among consumers.

Because of its origin and handling processes, lettuce salad is recognized as one of the dishes with the highest rate of contamination by various microorganisms. The fermented acetic acid commercially known as vinegar can be used as an ingredient to add flavor to salads, but it is also known as a safe substance (Generally Recognized As Safe - GRAS) and can be used to decontaminate vegetables.

Vegetables consumed raw are responsible for outbreaks of foodborne illnesses caused by bacteria like: *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes*, usually found in the soil, *Salmonella*, *Shigella*, *Escherichia coli* and *Campylobacter* spp., found in the GI tract of animals, including humans. In general, contamination of raw fruit and vegetables occurs through contact with feces, sewage or untreated irrigation water[4].

Raw lettuce, due to its high contamination rate, can have several effects on human health, resulting in mild and self-limiting to severe diarrhea with severe dehydration, weight loss and anemia[5].

Several studies have shown that numerous outbreaks of foodborne diseases are related to the consumption of lettuce contaminated primarily by enterohemorrhagic *E. coli*[6,7,8,9], *Salmonella* spp.[10,11] and *Yersinia* spp.[12,13].

For the preparation of these vegetables, they must be washed with drinking water so that their microbial load can be reduced by 90%. However, this may not be enough to eliminate contamination, and the application of a sanitary plan is essential[14].

In Brazil, chlorine is the most commonly used product for decontamination of vegetables, particularly sodium hypochlorite, due to its low cost. The legal use recommendation is 200-250 ppm with minimum contact for 15 min[15].

In recent years, there has been an increase in the consumption of minimally processed fruits and vegetables. However, chlorinated chemicals used as sanitizers can form carcinogenic compounds with adverse impacts on human health, and new sanitizers and technologies need to be introduced to ensure the safety of these types of food[16].

Acetic acid has been studied for its efficiency in removing pathogens from fresh fruits and vegetables. A study on the disinfection of lettuce inoculated with *E. coli* O157:H7(10^7 $CFUg^{-1}$) evidenced that a commercially available rice vinegar with 5% acetic acid (pH 3.0) for 5 min reduced the population of this pathogen by three logarithmic cycles[17].

Commercial use of organic acid solutions can minimize environmental and public health risks[18]. There are also advantages in the use of these products because they are cheap, biodegradable and easy to handle[14]. The use of GRAS organic acids is recognized as safe although their efficacy depends on the type of food in question and varies from one organic acid to another[19].

There are several studies on the efficacy of these agents in fruits and vegetables, but most have been conducted under conditions of artificial contamination using various microorganisms. Additionally, the technical application conditions are not always comparable[17,20,21,22,23,24,25].

This research was conducted to evaluate the quality of lettuce salads (*Lactuca sativa*) marketed in self-service restaurants and the efficacy of decontamination through fermented acetic acid with different acidities and concentrations for mesophilic aerobic bacterial count, coliforms (total and thermotolerant) and *Salmonella* spp.

METHOD

Sample collection

The samples were composed of ready-to-eat, dressing-free lettuce salads (*Lactuca sativa*), displayed in self-service counters of four randomly selected restaurants identified as A, B, C and D, located in the city of Duque de Caxias, state of Rio de Janeiro, Brazil. The samples were aseptically collected in aluminized cardboard packages in the amount of approximately 350 g, identified and transported to the microbiological food control laboratory of the Grande Rio University (Unigranrio), where the analyses were performed. The apple-based acetic acids used in

this experiment were purchased in commercial establishments according to their acidity informed on the label.

Sample handling

From each sample, 14 subsamples of 25 g were obtained, of which seven were used to perform viable mesophilic aerobic bacterial and coliform (total and thermotolerant) count analyses, whereas the other seven were used to search for *Salmonella* spp. Of the 14 subsamples, two were analyzed separately for initial microbiota evaluation without treatment with fermented acetic acid; both were defined as control. The 12 subsamples were then subjected to decontamination with fermented acetic acid under immersion for 15 min at different proportions of volatile acidity (in g. $100mL^{-1}$) and diluted in sterile distilled water in the following solutions: acidity 4 to 10% and 50%, acidity 5 to 10% and 50%; and acidity 6 to 10% and 50%.

Microbiological analysis

The analyses were performed in triplicate to estimate the most probable number (MPN) of coliforms (total and thermotolerant), the presence/absence of *Salmonella* spp. and the total count of mesophilic aerobic bacteria. These analyses were performed as established by Normative Instruction n. 62 of August 26, 2003[26], and the results were compared to Resolution of the Collegiate Board (RDC) n. 12 of January 2, 2001, the regulatory standard of the Brazilian National Agency of Health Surveillance (Anvisa)[27].

Counting techniques were performed from the three consecutive serial decimal dilutions, in which the first dilution was made from the 25 g subsample and then added to 225 mL of 0.1% peptone saline solution. The next decimal dilutions were made from 1 mL of the first dilution in 9 mL of 0.1% peptone saline solution, followed by homogenization.

Coliform count - presumptive test

From each decimal dilution, a 1 mL aliquot was transferred to three sets of three Lauril Sulfate Tryptose (LST) broth tubes with inverted Durham tubes, which were incubated at 36 ± 1° C for 48 h. Turbidity, combined with the presence of gas in one or more Duhram tubes, characterized positivity.

Total coliform count - confirmatory test

Aliquots of each tube considered positive in the presumptive test were transferred by a bacteriological loop to tubes with Brilliant Green Bile Lactose broth (BGBL), with inverted Duhram tubes incubated at 36 ± 1ºC for 48 h. Results were expressed as log MPN/mL after comparison in a table.

Total thermotolerant coliform count - confirmatory test

Similarly, from each LST tube considered positive in the presumptive test aliquots were transferred by bacteriological loop to tubes with *Escherichia coli* (EC) broth and tubes with BGBL broth, with inverted Duhram tubes incubated at 45º C for 24 h. By comparison with the table, the results were expressed as log MPN/mL.

Total mesophilic aerobic bacterial count

The pour plate technique was applied by using 1.0 ml inoculum of each of the serial decimal dilutions in duplicate Petri dishes with standard pre-fused counting agar. After homogenization and solidification, the plates were inverted and incubated for 48 h at 36 ± 1ºC. Counts were performed in a colony counter for plates after the selection of plates with counts between 25 and 250 colony forming units (CFU). The results were expressed as log CFU/g of the sample.

Search for Salmonella spp.

First, we performed the pre-enrichment step, in which each 25 g aliquot of the lettuce salad subsample was added to 225 mL of buffered peptone water and incubated at 36 ± 1° C for 20 h. Then 0.1 ml aliquots were transferred to tubes containing Rappaport Vassiliadis broth. In parallel, 1 mL aliquots were also transferred to tubes containing cystine selenite broth and incubated in a water bath at 42° C for 24 h, in what was called the enrichment step. Next, growth aliquots were subjected to surface scattering in solid selective media to obtain isolated colonies using plates with Ramback Agar, Bismuth Sulfite, Xylose Lysine Deoxycholate (XLD) and *Salmonella-Shigella*. The plates were incubated at 36° C ± 1° C for 24 h. Three to five suspected colonies per plate were selected and inoculated on standard agar, incubated at 36 ± 1º C for 24 h for subsequent biochemical testing to verify typical behavior of *Salmonella* spp., absence of urease, typical biochemical reactions on TSI agar, lysine decarboxylation or not, biochemical and motility reactions on SIM agar and paper tape oxidase test. All tests were incubated at 36 ± 1° C for 24 h.

Statistical analysis was performed using Pearson's correlation coefficient, Tukey's t-test and PAST software. For all analyses we considered the significance level of 5%[28].

RESULTS AND DISCUSSION

Mesophilic aerobic bacterial counts in the control lettuce salad samples of the restaurants ranged from four to six log CFU/g (Table 1). Current legislation does not provide for limits on the total count of mesophilic aerobic bacteria in fresh vegetables, but, according to Morton[29], food with total aerobic microorganism counts above 6 log CFU/g begins to show signs of deterioration. One of the samples presented this high count characterizing possible deterioration.

Fermented acetic acid was found to be efficient at all concentrations and acidities ($p < 0.05$) to reduce mesophilic aerobic bacteria (Table 1). We could observe greater efficiency in the treatment with fermented acetic acid at 50%, with results of reduction of three to almost five decimal cycles in the samples submitted to this treatment and varying from two to more than four decimal cycles in the samples treated with 10% of fermented acetic acid. In this regard, the results are similar to those obtained by Entani et al.[30], who obtained reduction of three logarithmic cycles with the use of 2.5% fermented acetic acid. In Brazil,

similarly, Oliveira[31] achieved a reduction in the mesophilic aerobic bacteria count of up to two logarithmic cycles in the decontamination of lettuce with 2% and 20% vinegar for 15 min.

In acidities 5 and 6 (in g. 100 mL^{-1}), we obtained reduction results of almost five decimal cycles, while in the other samples the reductions obtained results where some reached up to four decimal cycles. However, statistically, there was no significant difference between treatments with fermented acetic acid in terms of acidity and concentrations, but there was a significant difference ($p < 0.05$) regarding the treatment with fermented acetic acid of the control samples, which denotes that the use of fermented acetic acid can be effective to reduce bacteria regardless of its concentration and acidity.

In Brazil, similarly, using 4% vinegar in kale decontamination, Silva[32] obtained significant reduction to acceptable levels in the count of mesophilic aerobic bacteria and total and thermotolerant coliforms.

In line with that, Zerio-Egli et al.[33] found that 1.6% and 2.5% acetic acid solutions used for 30 sec, 2 and 5 min, respectively, acted on the decontamination of green leaves with reductions between 1.5 and 4.0 log CFU/g of aerobic bacteria, mold and yeast and coliforms. They concluded that the reductions were not dose or time dependent. In the present study, we found that fermented acetic acid in the concentrations we used caused the bacterial reduction.

According to the International Commission on Microbiological Specifications for Foods (ICMSF)[21], total lettuce coliform counts are used as parameters of the present microbial load. Although they are not used as a legal basis, they work as a warning about the conditions of hygiene during handling and the risks to consumer health.

Total coliform count results in the control samples ranged from 0.47 to 2.66 log MPN/g (Table 2). The use of fermented acetic acid reduced MPN values of total coliforms in all treatments, mainly in samples from restaurants A and C, with some results even superior to two decimal cycles. In samples from restaurants B and D, low total coliform counts were found. As for the effect of the concentration, the reduction became more evident in treatments with 50% concentration of fermented acetic acid. Regarding acidity, the treatment with acidity 5 was the most efficient in both concentrations (10% and 50%), even reaching values above two decimal cycles. Oliveira[26] also found a reduction in the total coliform count from two to three logarithmic cycles in lettuce decontamination with 2% and 20% vinegar for 15 min. There was no significant difference between treatments with fermented acetic acid in terms of acidity and concentrations, but there was a significant difference ($p < 0.05$) regarding the treatment with fermented acetic acid in relation to the control samples.

For fecal coliforms, Anvisa's federal legislation[13] set a maximum limit of 2×10^2 MPN/g or 2.30 log MPN/g. According to Table 3, lettuce salad samples from restaurants A and B were considered unfit for consumption according to the legislation. Higher bacterial reduction efficiency was observed in these samples after the treatment with fermented acetic acid, since they presented a larger amount of coliforms, therefore, there was greater efficiency, with the reduction of two to almost three decimal cycles.

As for fecal coliforms, there was no significant difference between treatments with fermented acetic acid in terms of acidity and concentrations, but there was a significant difference

Table 1. Results (in arithmetic mean) of the use of fermented acetic acid at different concentrations and acidities in the decontamination of lettuce salad samples for the analysis of mesophilic aerobic bacteria count (in log CFU/g).

Restaurant	Control	Fermented acetic acid					
		Acidity 4		Acidity 5		Acidity 6	
		10%	50%	10%	50%	10%	50%
A	5.07	3.11	2.04	3.07	2.11	3.43	2.51
B	6.08	3.57	2.04	2.79	1.54	3.57	1.70
C	4.69	1.17	1.00	1.00	1.00	1.00	1.00
D	5.49	2.14	1.60	2.53	1.30	1.00	1.65

Table 2. Results of the use of fermented acetic acid at different concentrations and acidities in the disinfection of lettuce salad samples for the analysis of total coliforms count (in log MPN/g).

Restaurant	Control	Fermented acetic acid					
		Acidity 4		Acidity 5		Acidity 6	
		10%	50%	10%	50%	10%	50%
A	2.66	0.84	0.47	0.47	0.47	0.56	0.47
B	0.85	0.55	0.47	0.47	0.47	0.56	0.47
C	2.38	0.60	0.47	0.47	0.47	0.95	0.60
D	0.85	0.47	0.47	0.47	0.47	0.47	0.47

Table 3. Results of the use of fermented acetic acid at different concentrations and acidities in the disinfection of lettuce salad samples for the analysis of fecal coliforms (in log MPN/g).

Restaurant	Control	Fermented acetic acid					
		Acidity 4		Acidity 5		Acidity 6	
		10%	50%	10%	50%	10%	50%
A	2.66	0.48	0.48	0.48	0.48	0.48	0.48
B	3.04	1.18	0.48	0.48	0.48	0.48	0.48
C	0.95	0.48	0.48	0.48	0.48	0.48	0.48
D	0.48	0.48	0.48	0.48	0.48	0.48	0.48

($p < 0.05$) regarding the treatment with fermented acetic acid in relation to the control samples.

Using different types of fermented acetic acid, Entani et al.[30] found strong bacteriostatic action in 34 bacterial strains and also found bactericidal activity in enteropathogenic and enterohemorrhagic *E. coli*, considering that the latter is very resistant to acidity. The reduction observed in the amount of fecal coliforms in the present research also indicated the efficiency of fermented acetic acid.

Similarly, Amoah et al.[35] found that the use of vinegar as lettuce sanitizer at concentrations of approximately 33% and 17%, respectively for 1 min and for over 10 min, removed values greater than four logarithmic cycles of fecal coliforms. In the present research, the results obtained for the reduction of fecal coliforms were similar in the 10% and 50% concentrations of fermented acetic acid.

Chang and Fang[17], when treating inoculated lettuce (10^7 CFUg^{-1}) with commercial vinegar with 5% acetic acid (pH 3.0) for 5 min, found a reduction of three logarithmic cycles in the population of enterohemorrhagic *E. coli*. Similarly, Poimenidou et al.[23] demonstrated that, compared to other natural chemical and antimicrobial agents, vinegar had the highest lethal activity against enterohemorrhagic *E. coli*, with a reduction of 1.8-2.3 log CFU/g and 2.8 log CFU/g in the total bacterial count. Akbas and Ölmez[18] achieved a reduction of enterohemorrhagic *E. coli* between 1.3 and 1.7 log CFU/g in chopped lettuce using acetic acid between 0.5% and 1.0% for 2 and 5 min. Similarly, in the present study, a reduction greater than two logarithmic cycles was obtained in samples from two restaurants.

No *Salmonella* spp. was detected in any of the lettuce samples from the four restaurants. These results classify the analyzed samples as in accordance with the Anvisa RDC[22] that requires the absence of *Salmonella* spp. in 25 g of product for fresh vegetables, so as to protect public health.

Sengun and Karapinar[36] found a significant reduction of *Salmonella* Typhimurium in carrots using 4% grape vinegar with a reduction of 1.87 to 3.33 logarithmic cycles, depending on treatment time.

Nascimento et al.[37] reported that vegetables consumed raw are susceptible to microbiological contamination, since they do not undergo any heat treatment. Therefore it is important to disinfect them to reduce the number of microorganisms.

According to Azeredo, Conceição and Stamford[38], 100% of the raw salad samples analyzed in a university restaurant were within the standards required by current legislation, although some mesophilic aerobic counts were found in some samples, which also occurred in the present research. This shows that, even though *Salmonella* was not detected, establishments must comply with the hygiene and handling practices that involve the product.

Corroborating its action, the use of acetic acid at concentrations of 1 to 2% reduced the populations of enterohemorrhagic *Escherichia coli*, *Salmonella* Typhimurium and *Listeria monocytogenes* by 0.5% to 2.78 logarithmic cycles in lettuce and organic apples[39].

CONCLUSIONS

Although we did not find the presence of *Salmonella* spp., the samples collected in three restaurants were compromised in hygienic and sanitary terms due to the high presence of mesophilic aerobic bacteria and total coliforms, in addition to fecal coliforms above the legal standards. There was a statistical difference between control salads and those treated with apple-based acetic acid, regardless of concentrations and acidity, with significant reduction in counts of mesophilic aerobic bacteria, total and fecal coliforms. In non-compliant samples, legally accepted standards were restored due to the significant reduction in the amount of fecal coliforms. Fermented acetic acid has proven to be an efficient and easily accessible alternative for bacteriological decontamination of raw vegetable salads.

REFERENCES

1. Pezzuto A, Belluco S, Losasso C, Patuzzi I, Bordin P, Piovesana A et al. Effectiveness of washing procedures in reducing *Salmonella enterica* and *Listeria monocytogenes* on a raw leafy green vegetable (*Eruca vesicaria*). Front Microbiol. 2016;7:1663. https://doi.org/10.3389/fmicb.2016.01663
2. Sirsat SA, Neal JA. Microbial profile of soil-free versus in-soil grown lettuce and intervention methodologies to combat pathogen surrogates and spoilage microorganisms on lettuce. Foods. 2013;2(4):488-98. http://doi.org/10.3390/foods2040488

3. Behrens JH, Barcellos MN, Frewer LJ, Nunes TP, Franco BDGM, Destro MT et al. Consumer purchase habits and views on food safety: a brazilian study. Food Control. 2010;21(7):963-9. https://doi.org/10.1016/j.foodcont.2009.07.018
4. Beuchat LR, organizador. Surface decontamination of fruits and vegetables eaten raw: a review. Geneva: World Health Organization; 1998[acesso 2 out 2018]. Disponível em: http://apps.who.int/iris/bitstream/10665/64435/1/WHO_FSF_FOS_98.2.pdf
5. Adami AAV, Dutra MBL. Análise da eficácia do fermentado acético como sanitizante na alface (*Lactuca sativa*, L.). REAS. 2011;3;134-44.
6. Keskinen LA, Annous BA. Efficacy of adding detergents to sanitizer solutions for inactivation of *Escherichia coli* O157:H7 on romaine lettuce. Int J Food Microbiol. 2011;147(3);157-61. https://doi.org/10.1016/j.ijfoodmicro.2011.04.002
7. Slayton RB, Turabelidze G, Bennett SD, Schwensohn CA, Yaffee AQ, Khan F et al. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 associated with romaine lettuce consumption. Plos One. 2011;8(2):e55300. https://doi.org/10.1371/journal.pone.0055300
8. Hilborn ED. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch Intern Med. 1999;159(15):1758-64. https://doi.org/10.1001/archinte.159.15.1758
9. Marder EP, Garman KN, Ingram LA, Dunn JR. Multistate outbreak of *Escherichia coli* O157:H7 associated with bagged salad. Foodborne Pathog Dis. 2014;11(8):593-5. https://doi.org/10.1089/fpd.2013.1726
10. Gajraj R, Pooransingh S, Hawker J, Olowokure B. Multiple outbreaks of *Salmonella braenderup* associated with consumption of iceberg lettuce. Int J Environ Health Res. 2012;22(2):150-5. https://doi.org/10.1080/09603123.2011.613114
11. L'Ecuyer PB, Diego J, Murphy D, Trovillion E, Jones M, Sahm DF et al. Nosocomial outbreak of gastroenteritis due to *Salmonella senftenberg* clinical infectious diseases: an official publication of the infectious diseases society of America. Clin Infect Dis. 1996;23(4):734-42. https://doi.org/10.1093/clinids/23.4.734
12. Macdonald E, Heier BT, Nygard K, Torunn S, Cudjoe KS, Skjerdal T et al. *Yersinia enterocolitica* outbreak associated with ready-to-eat salad mix, Norway, 2011. Emerg Infect Dis. 2012;18(9);1496-9. https://doi.org/10.3201/eid1809.120087
13. Nuorti JP, Niskanen T, Hallanvuo S, Mikkola J, Kela E, Hatakka M et al. A widespread outbreak of *Yersinia pseudotuberculosis* O:3 infection from iceberg lettuce. J Infect Dis. 2004;189(5):766-74. https://doi.org/10.1086/381766
14. Frank JF, Takeushi K. Direct observation of *E. coli* O157:H7 inactivation on lettuce leaf using confocal scanning laser microscopy. In: Proceedings of International Conference of International Committee on Food Microbiology and Hygiene; Vendhoven, UK.
15. Secretaria de Saúde de São Paulo. Portaria CVS Nº 6, de 10 de março de 1999. Aprova o regulamento técnico sobre os parâmetros e critérios para o controle higiênicosanitário em estabelecimentos de alimentos. Diário Oficial Estado. 12 mar 1999.
16. Singh, S, Ritesh W, Soberly M, Baby Z, Baite H, Mishra S et al. A review on postharvest management and advances in the minimal processing of fresh-cut fruits and vegetables. J Microbiol Biotech Food Sci. 2019;8(5):1178-87. https://doi.org/10.15414/jmbfs.2019.8.5.1178-1187
17. Chang JM, Fang T. Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. Food Microbiol. 2007;24(7-8):745-51. https://doi.org/10.1016/j.fm.2007.03.005
18. Akbas MY, Olmez H. Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. Lett App Microb. 2007;44(6):619-24. https://doi.org/10.1111/j.1472-765X.2007.02127.x
19. Figueiredo FF. Desinfeção de alfaces por ação do cloro e do vinagre e desenvolvimento de um sistema de segurança para alface em estabelecimentos de restauração coletiva [dissertação]. Lisboa: Universidade Técnica de Lisboa; 2013[acesso 20 mar 2013]. Disponível em: https://www.repository.utl.pt/handle/10400.5/5963
20. Nascimento M, Silva N, Catanozi MPLM, Silva KC. Avaliação comparativa de diferentes desinfetantes na descontaminação de uva. Braz J Food Technol. 2003;6(1):63-8.
21. Souza GC, Spinosa WA, Oliveira TCRM. Sanitizing action of triple-strength vinegar against *Escherichia coli* on lettuce. Hortic Bras. 2018;36(3):414-8. https://doi.org/10.1590/s0102-053620180321
22. Ramos B, Brandão TRS, Teixeira P, Silva CLM. Balsamic vinegar from Modena: an easy and effective approach to reduce *Listeria monocytogenes* from lettuce. Food Control. 2014;42:38-42. https://doi.org/10.1016/j.foodcont.2014.01.029
23. Poimenidou SV, Bikouli VC, Gardeli C, Mitsi C, Tarantilis PA, Nychas GJ et al. Effect of single or combined chemical and natural antimicrobial interventions on *E. coli* O157:H7, total microbiota and color of packaged spinach and lettuce. Int J Food Microbiol. 2016;220:6-18. https://doi.org/10.1016/j.ijfoodmicro.2015.12.013
24. Arvanitoyannis IS, Bouletis A, Papa EA, Gkagtzis DC, Hadjichristodoulou C, Papaloucas C. The effect of addition of olive oil and "aceto balsamico di Modena" wine vinegarin conjunction with active atmosphere packaging on the microbial T and sensory quality of "lollo verde" lettuce and rocket salad. Anaerobe. 2011;17(6):303-6. https://doi.org/10.1016/j.anaerobe.2011.04.010
25. Vijayakumar C, Wolf-Hall CE. Minimum bacteriostatic and bactericidal concentrations of household sanitizers for *Escherichia coli* strains in tryptic soy broth. Food Microbiology. 2002;19(4):383-8. https://doi.org/10.1006/fmic.2002.0459

26. Ministério da Agricultura, Pecuária e Abastecimento (BR). Instrução Normativa Nº 62, de 26 de agosto de 2003. Oficializa os métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água. Diário Oficial União. 18 set 2003.

27. Agencia Nacional de Vigilância Sanitária - Anvisa. Resolução RDC Nº 12, de 2 de janeiro de 2001. Dispõe sobe regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial União. 2 jan 2001.

28. Hammer O, Harper DAT, Ryan PD. Paleontological statistics software package for education and data: analysis. Paleo Electronica. 2001;4(1):1-9.

29. Morton RD. Aerobic plate count. In: Downes FP, Ito K, editores. Compendium of methods for the microbiological examinations of foods. Washington: American Public Health Association; 2001. p. 63-6.

30. Entani E, Asai M, Tsujihata S, Tsukamoto Y, Ohta M. Antibacterial actin of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7 Part 1: examination of bacteriostatic and bactericidal activities. Kansenshogaku Zasshi. 1997;71(5):443-50.

31. Oliveira ABA. Comparação de diferentes protocolos de higienização de alface (*Lactuca sativa*) utilizados em restaurantes de Porto Alegre – RS [dissertação]. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2005[acesso 19 set 2018]. Disponível em: http://hdl.handle.net/10183/5885

32. Silva APG. Eficácia da água sanitária e do vinagre como sanitizantes de couve (*Brassica oleracea* L.) [monografia]. Recife: Universidade Federal de Pernambuco; 2016.

33. Zerio-Egli CN, Sujata AS, Neal JA. Development of a novel economical device to improve post-harvest processing practices on small farms. Food Control. 2014;44:152-8. https://doi.org/10.1016/j.foodcont.2014.03.057

34. International Commission on Microbiological Specification for Foods – ICMSF. International Union of Microbiological Societies - IUMS. APCC na qualidade e segurança microbiológica de alimentos: análise de perigos e pontos críticos: qualidade e segurança microbiológica de alimentos. São Paulo: Varela; 1997[acesso 14 fev 2018]. Disponível em: http://www.icmsf.org/pdf/FSO%20Ojectives/GuiaSimplificadoPO.pdf

35. Amoah P, Drechsel P, Abaidoo RC, Klutse A. Effectiveness of common and improved sanitary washing methods in selected cities of West Africa for the reduction of coliform bacteria and helminth eggs on vegetables. Trop Med Int Health. 2007;12(supl2):40-50. https://doi.org/10.1111/j.1365-3156.2007.01940.x

36. Sengun IY, Karapinar M. Effectiveness of lemon juice, vinegar and their mixture in the elimination of *Salmonella typhimurium* on carrots (Daucus carota L.). Int J Food Microbiol. 2004;96(3):301-5. https://doi.org/10.1016/j.ijfoodmicro.2004.04.010

37. Nascimento WM, Cantliffe DJ, Huber DJ. Seed aging affects ethylene production and endo-b-mannanase activity during lettuce seed germination at high temperature. Seed Sci Tech. 2005;33(1)11-17. https://doi.org/10.15258/sst.2005.33.1.02

38. Azerêdo GA, Conceição ML, Stamford TLM. Qualidade higiênico-sanitária das refeições em um restaurante universitário. Hig Alim. 2004;18(25):74-8.

39. Park SH, Choi MR, Park KH, Chung MS, Ryu S, Kang DH. Use of organic acids to inactivate *Escherichia coli* O157:H7, *Salmonella Typhimurium*, and *Listeria monocytogenes* on organic fresh apples and lettuce. J Food Sci. 2011;76(6):293-8. https://doi.org/10.1111/j.1750-3841.2011.02205.x

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.