

**Special Issue (UniSZA), Malaysia:**ICAFT 2023: 3rd International Conference on Agriculture, Animal Sciences & Food Technology

Effect of Different Conditions of Citric Acid and Acetic Acid Decontamination against *Listeria Monocytogenes* in Lettuce

Wan Norhafizah Wan Azman and John Yew Huat Tang*

Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (Besut Campus), 22200 Besut, Terengganu, Malaysia.

Abstract | The purpose of this study was to assess the effectiveness of various citric and acetic acid conditions against *L. monocytogenes* in lettuce, as well as to ascertain the overall reduction of *L. monocytogenes* by various decontamination procedures. Before decontamination process, the sample was inoculated with *L. monocytogenes* and stored in the chiller for 24 h. Different concentrations of acetic and citric acid (0%, 0.5%, 1.0%, 1.5%, and 2.0%), as well as varied times (0, 15, and 30 min) and physical applications (agitation and without agitation) were used to clean the sample. After being incubated for 48 h at 30°C, the numbers of *L. monocytogenes* was counted. It has been demonstrated that at a concentration of 1.5%, citric acid is efficient in eliminating *L. monocytogenes* without agitation. With a citric acid (1.0%), the effectiveness of citric acid in killing *L. monocytogenes* is greatly enhanced by the use of physical forces. The duration of the citric acid treatment was also found to have a major impact on the elimination of *L. monocytogenes* in lettuce. Citric acid application for 30 min was shown to be significantly more efficacious than that for fifteen minutes ($p < 0.05$). On the other hand, at a concentration of 2.0%, the results indicated that acetic acid is highly effective in eliminating *L. monocytogenes* without the need for physical application. However, with agitation, it exhibits a notable decrease at the minimal concentration of 0.5%. The amount of *L. monocytogenes* does not, however, significantly change when the period is extended from 15 to 30 min. To summarize, when physical force is applied, acetic acid is more effective than citric acid; nevertheless, without agitation, the data demonstrate that citric acid is more effective than acetic acid.

Received | February 22, 2024; **Accepted** | August 06, 2024; **Published** | October 07, 2024***Correspondence** | John Yew Huat Tang, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin; **Email:** jyhtang@unisza.edu.my**Citation** | Azman, W.N.W. and J.Y.H. Tang. 2024. Effect of different conditions of citric acid and acetic acid decontamination against *Listeria monocytogenes* in lettuce. *Sarhad Journal of Agriculture*, 40(Special issue 1): 22-27.**DOI** | <https://dx.doi.org/10.17582/journal.sja/2024/40/s1.22.27>**Keywords** | Acetic acid, Citric acid, *Listeria monocytogenes*, Lettuce, Time, Concentration**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

The pathogenic bacteria that causes listeriosis in humans is called *Listeria monocytogenes*. Being facultative anaerobic, the bacteria may live in both oxygen-rich and oxygen-deficient environment.

It represents an important pathogen because *L. monocytogenes* can tolerate osmotic stress and persist at temperatures below freezing (Rhoades *et al.*, 2009). Food contaminated with *L. monocytogenes* can cause listeriosis, a potentially fatal infection. It is a significant public health hazard in the US, where an

estimated 1,600 individuals get critically ill from it every year, leading to 260 deaths, according to the US Centers for Disease Control and Prevention (CDC) (Rogalla and Bomar, 2023).

According to Oliver *et al.* (2005) and Rahimi *et al.* (2010), listeriosis is linked to consumption of tainted milk, soft or semi-soft cheese, undercooked, ready to eat foods, and unwashed raw fruits and vegetables. The condition primarily affects elderly people, pregnant women, infant, and adults with compromised immune systems. People who are not in these groups are less likely to become ill from it, but they can be impacted on occasion. According to the CDC, infected people may have fever, muscle aches, and gastrointestinal symptoms such as nausea or diarrhea.

Fruits and vegetables are a key part of a healthy diet, which is why there is a global push to boost their consumption (FAO/WHO, 2005). Globally, people are becoming more health conscious these days, and they tend to eat more veggies. Given that vegetables are frequently eaten in their least processed state, the public would be concerned if *L. monocytogenes* was found in them (Dogbe, 2010). Foods classified as minimally processed are those that are produced, consumed raw, and have not undergone any formal processing or treatment to lower the levels of harmful bacteria, their spores, or their toxins. The purpose of this study is to examine the effects of different citric acid and acetic acid concentrations, application methods, and time duration on decontamination against *L. monocytogenes* on lettuce.

Materials and Methods

Preparation of vegetables

The selected vegetable lettuce is purchased from the local hypermarket. Damaged layer, leaves and the lettuce core lettuce were removed and discarded. The selected leaves were then cut into circular pieces with a diameter of 4 cm each using sterile scalpel. The leaves were then placed in plastic Petri dish.

Preparation of L. monocytogenes inoculums

The strain of *L. monocytogenes* (ATCC 13932) was grown in the 10 ml tryptic soy broth (Merck, Germany) for 24 h at 30°C. The culture was poured into the eppendorf tube and centrifuged using the centrifuging machine (5000 rpm, 10 min, Eppendorf Centrifuge 5418). Cell pellets was resuspended in

saline solution, and measured the optical density using the spectrophotometer with wavelength of 600 nm. The *L. monocytogenes* inoculum was set at absorbance 1.949 equals to approximately 6.0×10^9 cfu/ml.

L. monocytogenes inoculated on the salad vegetables

A 0.1 ml of the washed bacterial culture of concentration 10^9 cfu/ml was inoculated onto the upper surface of the lettuce. The inoculum of *L. monocytogenes* was spot-inoculated in 6-8 droplets and then spread around using the pipette tip for the entire surface to be covered as evenly as possible. While spreading the inoculums, the cut edge of the vegetables was avoided. Then, the inoculum was allowed for contact with the vegetables for 24 h and kept at 4°C.

Decontamination of vegetables

The decontamination methods that were conducted are:

1. Dipping the lettuce into the acetic acid solution with different concentration and time (without agitation)
2. Dipping the lettuce into the citric acid solution with different concentration and time (without agitation)
3. Dipping the lettuce in the acetic acid solution with different concentration and time (with agitation)
4. Dipping the lettuce in the citric acid solution with different concentration and time (with agitation)

Using the samples that are incubated earlier, the decontamination experiments was carried out to investigate the effect of the different decontamination methods on the reduction of the *L. monocytogenes*.

The acetic acid solution and citric acid solution of concentration 0.5%, 1.0%, 1.5% and 2.0% v/v was prepared. The pH of the two solutions with different concentration was measured using the pH meter (CRISON microPH2001). Then, the samples that are incubated earlier were treated with the different solution of different concentration, time and physical application. The vegetable was immersed in the solution with different parameters in the beaker. For the treatment with agitation, the vegetable was immersed in the solution and agitated with the magnetic stirrer hot plate. Lastly, the vegetables samples were drained and placed in sterile bag that contain saline solution to dilute the remaining acid residue on the vegetables.

Enumeration of *L. monocytogenes*

The vegetables samples were transferred into the stomacher bag containing 50 ml saline solution and homogenized for 30s by mixing the solution with samples. The serial dilutions were done using the homogenized samples (0.1 ml) until 10^{-4} cfu/ml. For the enumeration of *L. monocytogenes*, the samples from the serial dilution were spread on the surface of the PALCAM agar (Merck, Germany). The plates were incubated at 30°C for 48 hours. The colony counts will be transformed to log cfu/cm² for the vegetable samples. While the uninoculated samples were also stored under the same condition (30°C and 48 h) and was analyzed for the presence of the *L. monocytogenes*.

Data analysis

The microbial counts were transformed to the logarithms before calculating the means and standard deviations. The population densities are using log cfu/cm² for the vegetables sample. Thus, the population densities of *L. monocytogenes* at the end result were compared to determine the effectiveness of the decontamination methods. The data from the analysis were evaluated using the Tukey's post-hoc test (SPSS). The significance level for all tests is 0.05.

Results and Discussion

Kim *et al.* (2011) stated that lettuce is one of the most widely consumed vegetables that undergoes minimal processing, meaning it is typically consumed raw. It is challenging to guarantee the safety and quality elements of the vegetables throughout production because the minimally processed vegetables are not subjected to harsh preservation methods (Kondo *et al.*, 2006).

Table 1 shows the outcome of the study on the impact of time and citric acid solution concentration on the survival of *L. monocytogenes* inoculation on salad vegetable surfaces. *L. monocytogenes* was not detected in all the uninoculated lettuce samples.

The *L. monocytogenes* colonies were counted 24 h after being incubated at 30°C in the incubator. An inoculum size of 6.48 log cfu/cm² of *L. monocytogenes* were recovered on the PALCAM agar. Sterile distilled water without citric acid serve as control throughout the study. Table 1 summarized the result of citric acid as decontamination agent for lettuce artificially contaminated with *L. monocytogenes*. Physical force of

agitation have shown effectively reduce the numbers of *L. monocytogenes* even without the use of citric acid. Decontamination with agitation in sterile water showed significant reduction of *L. monocytogenes* to 4.55 log cfu/cm² and 4.24 log cfu/cm² after 15 min and 30 min, respectively.

Table 1: The effect of the different concentration of citric acid (0, 0.5, 1.0, 1.5, and 2.0%), time (0, 15, and 30 min) and the method (agitate, without agitate) on lettuce inoculated with *L. monocytogenes* (log cfu/cm²).

Concentration (%)	Time		
	0 min	15 min	30 min
Agitation			
0.0	6.48±0.00 ^{aA}	4.55±0.04 ^{bA}	4.24±0.23 ^{bA}
0.5	6.48±0.00 ^{aA}	2.95±0.92 ^{bB}	0.00±0.00 ^{cB}
1.0	6.48±0.00 ^{aA}	0.00±0.00 ^{bC}	0.00±0.00 ^{bB}
1.5	6.48±0.00 ^{aA}	0.00±0.00 ^{bC}	0.00±0.00 ^{bB}
2.0	6.48±0.00 ^{aA}	0.00±0.00 ^{bC}	0.00±0.00 ^{bB}
Without agitation			
0.0	6.48±0.00 ^{aA}	5.94±0.33 ^{abA}	5.72±0.00 ^{bA}
0.5	6.48±0.00 ^{aA}	5.70±0.61 ^{abA}	4.83±0.59 ^{abB}
1.0	6.48±0.00 ^{aA}	4.95±0.95 ^{abB}	4.29±1.32 ^{bB}
1.5	6.48±0.00 ^{aA}	0.00±0.00 ^{bC}	0.00±0.00 ^{bC}
2.0	6.48±0.00 ^{aA}	0.00±0.00 ^{bC}	0.00±0.00 ^{bC}

^{a, b, c} different small letters within row indicate significant difference ($p < 0.05$). ^{A, B, C} different capital letters within column indicate significant difference ($p < 0.05$).

Fifteen minutes decontamination duration capable of removing all the pathogen with citric acid with concentration 1.0% and above. Concentration of 0.5% citric acid reduce the numbers to 2.95 log cfu/cm². Longer treatment duration (30 min) proved to increase the effectiveness in removing *L. monocytogenes* from lettuce with citric acid (0.5%) significantly remove all the inoculated *L. monocytogenes*. Soaking lettuce without agitation, *L. monocytogenes* was significantly reduced after 30 mins in control, 0.5, and 1.0% of citric acid to 5.72, 4.83 and 4.29 log CFU/cm², respectively. Citric acid concentration of 1.5 and 2.0% significantly reduced the number of *L. monocytogenes* after 15 mins to no detectable level. Based on the result, the agitation plays an important role in reducing the numbers of *L. monocytogenes* at a higher number and shorter time.

The effects of acetic acid treatment on the inactivation of *L. monocytogenes* on the lettuce are shown in the Table 2. The number of *L. monocytogenes* was reduced

significantly after the samples are treated with 0.5% acetic acid for 15 min with agitation. Increasing the treatment time from 15 to 30 min did not show any significant ($p < 0.05$) reduction in the number of *L. monocytogenes*. Increasing acetic acid concentration to 1.0, 1.5 and 2.0% significantly reduced the numbers to non-detectable level after 15 mins of treatment.

Table 2: The effect of the different concentration of citric acid (0%, 0.5%, 1.0%, 1.5%, 2.0%), time (0, 15, and 30 min) and the method (agitate, without agitate) on lettuce inoculated with *L. monocytogenes* (log cfu/cm²).

Concentration (%)	Time		
	0 min	15 min	30 min
Agitation			
0.0	6.41±0.08 ^{aA}	4.47±0.66 ^{bA}	4.32±0.17 ^{bA}
0.5	6.41±0.08 ^{aA}	0.00±0.00 ^{cB}	0.00±0.00 ^{cB}
1.0	6.41±0.08 ^{aA}	0.00±0.00 ^{cB}	0.00±0.00 ^{cB}
1.5	6.41±0.08 ^{aA}	0.00±0.00 ^{bB}	0.00±0.00 ^{bB}
2.0	6.41±0.08 ^{aA}	0.00±0.00 ^{bB}	0.00±0.00 ^{bB}
Without agitation			
0.0	6.41±0.08 ^{aA}	6.68±0.66 ^{aA}	6.35±0.11 ^{aA}
0.5	6.41±0.08 ^{aA}	4.37±0.59 ^{bB}	4.30±0.28 ^{bB}
1.0	6.41±0.08 ^{aA}	4.48±0.30 ^{bB}	4.39±0.16 ^{bB}
1.5	6.41±0.08 ^{aA}	5.84±0.07 ^{bB}	4.77±1.09 ^{bB}
2.0	6.41±0.08 ^{aA}	0.00±0.00 ^{bC}	0.00±0.00 ^{bC}

^{a, b, c} different small letters within row indicate significant difference ($p < 0.05$). ^{A, B, C} different capital letters within column indicate significant difference ($p < 0.05$).

Treatment without agitation showed no significantly reduction in control samples after 15 and 30 mins. However, significantly reduction was seen with 15 mins of treatment with 0.5, 1.0, 1.5 and 2.0% acetic acids with 4.37, 4.48 and 5.84 log CFU/cm², respectively. Increasing the treatment duration to 30 mins did not significantly reduced the *L. monocytogenes* numbers. Acetic acid with 2.0% reduced the *L. monocytogenes* to no detectable level.

According to Tirpanalan *et al.* (2011), organic acid is useful in lowering the number of *L. monocytogenes* because acetic and citric acids have antimicrobial qualities that can lower the pH of the solution and, through the dissociation of hydrogen ions, lower the internal cellular pH of the organism. The disruption of the cell's ability to maintain the internal cellular pH can have an impact on the permeability of the membrane and substrate transport (Betts and Elveris, 2005). Beuchat and WHO (2002) reported that

washing fruits and vegetables surfaces with organic acid may help lower the amount of microorganisms present. It has been demonstrated that simple procedures such as rubbing lemons, which contain citric acid, on sliced fruits can be effective to either eliminate or significantly slow the growth of bacteria. Other study also reported similar finding of lemon juice application to the surface of jicama and papaya that have been contaminated with *Salmonella typhi* reduces the numbers compared to the control group temporarily because the numbers increased after several hours (Escartin *et al.*, 1989). Another study found that treating salad greens with up to 1500 ppm of citric acid had no effect on the growth of bacteria over the four days that they were stored at 10°C (Shapiro and Holder, 1960).

Organoleptic changes may occur for vegetables treated with organic acids as shown by Akbas and İlmez (2009) that acetic acid change lettuce organoleptic properties at concentration greater than 1.0%. However, it is unclear whether citric acid can alter the organoleptic qualities of the vegetables. Thus, it is preferable to attain the greatest degree of microbial population decrease at the lowest concentration of organic acids with shortest treatment duration as a precaution to preserve the vegetables quality and safety. Therefore, the physical application (agitation) can be employed to increase the efficacy of the organic acid decontamination treatment.

The lipophilic qualities of the organic acids such as citric acid, acetic acid, malic acid, tartaric acid, sorbic acid, and lactic acid allow them to permeate the pathogens plasma membrane and acidify the inside of the cells (Booth *et al.*, 1989). Although organic acids are categorized as weak acids but they are capable of impeding microbial development more effectively than strong acids. Organic acids are generally recognised as safe (GRAS) for use in food treatments regardless the types of food thus making them safe for use by household to lower the risk of microbiological infection.

Conclusions and Recommendations

To sum up, organic acids like acetic and citric acid can effectively act as a decontamination agent against *L. monocytogenes* in lettuce to guarantee safe ingestion. In order to effectively eradicate *L. monocytogenes* from lettuce, two crucial elements are the concentration of

acid and the physical application while washing the vegetables.

Acknowledgements

This research was supported by Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin.

Novelty Statement

The current research work investigates effect of organic acids to remove *L. monocytogenes* from lettuce to ensure safe consumption of raw lettuce.

Author's Contribution

Wan Norhafizah Wan Azman and John Yew Huat Tang: Designed the study.

Wan Norhafizah Wan Azman: Performed the experiments and collected the data.

All the authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

References

- Akbas, M.Y. and H. Ölmez. 2009. Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. Lett. Appl. Microbiol., 44: 619-624. <https://doi.org/10.1111/j.1472-765X.2007.02127.x>
- Betts, G. and L. Elveris. 2005. Alternatives to hypochlorite washing systems for the decontamination of fresh fruit and vegetables. In: (W. Jongen). Improving the safety of fresh fruit and vegetables, Woodhead Publishing Limited and CRC Press, Cambridge, England. pp. 351-372. <https://doi.org/10.1533/9781845690243.3.351>
- Beuchat, L.R. and WHO, 2002. Surface decontamination of fruits and vegetables eaten raw: A review. Food Safe Issue World Health Organization, Genève. Available at http://apps.who.int/iris/bitstream/10665/64435/1/WHO_FSF_FOS_98.2.pdf (Accessed at 1 August 2024).
- Booth, I.R., R.G. Kroll and G.W. Gould. 1989. The preservation of foods by low ph. In: (ed. G.W. Gould). Mechanisms of action of food preservation procedures. Elsevier Applied Science, Elsevier Science Publishers Ltd. London, pp. 119-160.
- Dogbe, E.E., 2010. Risk of *Listeria monocytogenes* ingestion in consuming coleslaw purchased from food vendors in the Accra Metropolis. Master in Philosophy in the Department of Nutrition and Food Science, University of Ghana. Available at <https://core.ac.uk/reader/132642220> (Accessed at 1 August 2024).
- Escartin, E.F., A.C. Ayala and J.S. Lozano. 1989. Survival and growth of *Salmonella* and *Shigella* on sliced fresh fruit. J. Food Prot., 52(7): 471-472. <https://doi.org/10.4315/0362-028X-52.7.471>
- FAO/WHO, 2005. Fruit and vegetables for health. Report of a joint FAO/WHO Workshop, 1-3 September 2004, Kobe, Japan. Available at: https://iris.who.int/bitstream/handle/10665/43143/9241592818_eng.pdf?sequence=1&disAllowed=y (Accessed 1 August 2024).
- Kim, S.Y., D.H. Kang, J.K. Kim, J.Y. Ha, T. Kim and S.H. Lee. 2011. Antimicrobial activity of plant extracts against *Salmonella typhimurium*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on fresh lettuce. J. Food Sci., 76: 41-46. <https://doi.org/10.1111/j.1750-3841.2010.01926.x>
- Kondo, N., M. Murata and K. Isshiki. 2006. Efficiency of sodium hypochlorite, fumaric acid, and mild heat in killing native microflora and *Escherichia coli* O157:H7, *Salmonella typhimurium* DT104, and *Staphylococcus aureus* attached to fresh cut lettuce. J. Food Prot., 69: 323-329. <https://doi.org/10.4315/0362-028X-69.2.323>
- Oliver, S.P., B.M. Jayarao and R.A. Almeida. 2005. Food-borne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathog. Dis., 2: 115-129. <https://doi.org/10.1089/fpd.2005.2.115>
- Rahimi, E., M. Ameri and H. Momtaz. 2010. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. Food Contr., 21: 1448-1452. <https://doi.org/10.1016/j.foodcont.2010.03.014>
- Rhoades, J.R., G. Duffy and K. Koutsoumanis. 2009. Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella*

- enterica* and *Listeria monocytogenes* in the beef production chain: A review. Food Microbiol., 26: 357–376. <https://doi.org/10.1016/j.fm.2008.10.012>
- Rogalla, D. and P.A. Bomar. 2023. *Listeria monocytogenes*. Available at <https://www.ncbi.nlm.nih.gov/books/NBK534838/> (Accessed on 2 July 2024).
- Shapiro, J.E. and I.A. Holder. 1960. Effect of antibiotic and chemical dips on the microflora of packaged salad mix. Appl. Microbiol., 8(6): 341-345. <https://doi.org/10.1128/am.8.6.341-345.1960>
- Tirpanalan, O., M. Zunabovic, K. Domig and W. Kneifel. 2011. Mini review: Antimicrobial strategies in the production of fresh-cut lettuce products. In: (A.Méndez-Villas). Science against microbial pathogens: Communicating current research and technological advances. pp. 176-188. Available at: <https://www.semanticscholar.org/paper/Mini-review%3A-Antimicrobial-strategies-in-the-of-Tirpanalan-%C5%BDuna%20bovi%C4%87/2d0f11c8dd74dbf138e1577b7aa98c7ae38301f9> (Accessed on 6 July 2024).