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Effect of Organic Acid and Commercial Washing Solutions for Bacteria Removal from Lettuce Collected from Market in Phnom Penh

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Abstract: *Lettuce (Lactuca sativa) is a green leafy vegetable popularly consumed fresh without prior cooking, potentially posing a health risk if poorly handled. For this reason, washing is crucial for reducing contaminated microorganisms before consumption. There are many commercial washing solutions in the market. Yet, their effectiveness remains unclear. This study aimed to evaluate and compare the efficacies of different washing solutions, such as single-use of organic acid citric acid (CA) and acetic acid (AA), two commercial washing solutions (CWS-A and CWS-B) purchased from the market, and Deionized water (DW). The lettuce samples in this study were purchased from Kilo 4 market and Samaki markets in Phnom Penh on the day of an experiment by randomly selecting from different vendors. The acetic acid solution (v/v) and citric solution (w/v) were used to wash lettuce samples at concentrations of 1, 2, 4, and 5% ; CWS-A (citric acid, sodium chloride, lemon flavor, and water), and CWS-B (acid acetic, citric acid, sodium chloride and water). The viable bacteria count was enumerated on Luria Bertani agar to determine the concentration of viable bacteria before and after washing with the different washing solutions for 10 min at static conditions. Unwashed samples were used as a control. The average initial level of viable bacteria counts by unwashed lettuce sample was 6.14 log CFU/g. After washing for 10 min, the viable bacteria count showed that organic acid as acetic acid at 1, 2, 4, and 5% was reduced by 1.95, 2.79, 3.6, 3.7 log CFU/g, respectively, and citric acid at 1, 2, 4, 5% were 1.55, 1.73, 3.3, 3.6 log CFU/g, respectively. Furthermore, the result of a commercial product of CWA was reduced to 1.1 log CFU/g, and CWB was reduced to 1.39 log CFU/g. Among all washing solutions, organic acid as acetic and citric acid at 4 and 5% concentrations were more effective than other tested solutions for reducing viable bacteria count.*

Keywords: Viable Bacteria Count, Lettuce, Washing Solution, Bacterial Removal

1. INTRODUCTION

Vegetables play important part of the Cambodian diet and contribute heavily to human livelihoods and prosperity. Vegetables are Cambodia's second most important crop after rice. The most proportion of crops is produced in five provinces, including Kandal, Kampot, Takeo, Siem Reap, and Kompong Chhnang province [1]. The popular vegetables include cucumber, tomato, cabbage, and Chinese cabbage, which are among Cambodia's most consumed vegetables [2]. Lettuce (Lactuca sativa) is a type of leafy green vegetable that is commonly found in traditional dishes and is believed to provide many nutritional values, such as minerals, fiber, vitamins, and antioxidants that could support human metabolisms [3]. In many dishes, the lettuce is consumed fresh without any processing besides soaking and washing with water or sometimes with commercial vegetable washing solution. Although fresh lettuce has many health benefits, cross-contamination by pathogenic microorganisms throughout the food supply chain, such as bacteria, parasites, or fungi, could cause serious foodborne diseases. The high number of foodborne outbreaks associated with consuming

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contaminated vegetables worldwide has been observed [4,5]. According to in previous study in 2022, about 56.5% of lettuce and 34.9% of cucumber sold in the market were contaminated with Salmonella during the dry season in Cambodia [6]. Additionally, according to a previous study in Vietnam, lettuce sold in the market was contaminated with Aerobic bacteria, Escherichia coli, and Salmonella spp. was 6.99 log CFU, 5.82 log CFU, and 3.33% respectively [7].

Cross-contamination of fresh vegetables may occur through various routes from farm to fork. Somewhat the possible source of contamination includes microbial contamination of raw products, personal hygiene of food handlers, and the environment and equipment used in the salad and fresh vegetables processing and distribution. At the farm level, untreated contaminated water with animal manures or access to livestock or wild animals leads to microbial spoilage [8]. Several studies have documented high microorganism contamination at the packing step [9]. Besides that, distribution is the process of moving food from a farm or processing facility to a customer or an institution that provides food services, such as a cafeteria, restaurant, or kitchen [10]. Foods may need to be transported once during this stage, such as when produce is trucked from a farm to the local market. Fresh produce can be contaminated if loaded into a truck that was not cleaned. Moreover, the center for disease control (CDC) guidelines for the microbiological quality of ready-to-eat foods range from satisfactory to potentially hazardous. Sometimes, consuming fresh leafy vegetables without proper processing is a potential route to foodborne disease outbreaks [11].

Several bacterial reduction approaches in food products significantly inhibit the circulation of foodborne diseases [12]. In contrast, consumers' opinions on the decontamination techniques such as irradiation, ultrasound, and chemical sanitizers have recently been taken as novel methods but also cause various harmful impacts on human health and the environment [13]. Many European countries have already prohibited the application of chlorine as a chemical disinfectant. On top of that, bacterial regrowth is another issue that might reduce the efficacy of many sanitizers [14]. Local Cambodians and people worldwide usually use water for washing and rinsing fresh fruit and vegetable products. Based on a recent study, some fruits and vegetables benefit from a more extended shelf-life by reducing the bacterial contamination on their surfaces after washing and rinsing, but it may remove only a portion of pathogenic microorganisms [15]. Therefore, the antimicrobial efficiency of the washing formula or commercialized products such as organic acids, chemical agents, and washing solutions could be considered as the assurance for reducing or eliminating microorganisms in fresh products [16]. For this reason, as seen in Cambodia, this kind of study lacks the evaluation efficiency of commercial wash solutions sold in the market.

This study aims to evaluate the efficacy level of different organic acids, such as acetic and citric acid, and commercial products to reduce bacterial load in fresh lettuces products. This study provided knowledge on the safety status of fresh food products and can further develop as low-cost washing for eliminating harmful microorganisms in leafy vegetables before human consumption.

2. METHODOLOGY

2.1 Sampling

Lettuces samples were randomly purchased from vendors such as Kilo 4 market and Samaki markets in Phnom Penh city. After collection, the samples were packed in a plastic bag and immediately transported for microbiological analysis at the Environmental Microbiology laboratory of the Research and Innovation Center, located at the Institute of Technology of Cambodia.

2.2 Initial bacteria enumeration

After aseptically cutting into small pieces, 10 g of the sample was transferred into a sterile stomacher bag filled with 90 ml of phosphate buffer solution (PBS: 8 g of NaCl, 0.24 g of KCl, 1.44 g of Na2HPO4, 0.24 g of KH2PO 4 in 1 L of distilled water, then was sterilized by autoclave at 121 °C for 15 minutes). It was then hand massaged for 1 min to ensure homogenously suspend the microbial on the sample's surface into the buffer. Samples solution was diluted with PBS at 1:10 in a serial dilution. After that, 0.1 ml of the diluted solution was enumerated on the Luria Bertani agar plate (LB) (Becton, Dickinson & Company, USA). Finally, the bacterial viable count was determined on the plate after incubation for 24 h at 37 °C [17].

After washing, 10 g of the treated sample were septically cut and transferred into another sterilized stomacher bag containing 90 ml PBS solution before homogenizing by hand messaging for 1 min. Serial dilution was prepared from the original homogenate in PBS with a ratio of 1:10 (w/v). After the sample was diluted with final dilution factor 10-3; then 100µl of the sample diluted at 10-1 and 10-3; were spread onto LB agar plates. Finally, the plates were incubated at 37 °C for 24 hours.

2.2.1. Vegetable washing conditions

The sample is treated with three different conditions: deionized water, organic acids, and commercialized washing solutions. Two kinds of organic acids were used in the assay; acetic acid (Scharlab SL, Sentmenat, Spain), and citric acid (Weifang Ensign Industry Co., Ltd., Changle, China), which were used in this experiment. Acetic acid (1%, 2%, 4%, 5%) was prepared based on formular 1, and citric acid (1%, 2%, 4%) were prepared by weight the corresponding mass (Table 1). All prepared solutions were filtrated through a 0.45µm membrane filter to remove any contamination. In addition, the commercial washing solutions were purchased from different markets. First, the 5 ml of each solution was pumped by mixing with deionized water (1 L) into a sterile baker and mix it based on the manufacturer guideline. Finally, pH of the solution was measured.

Table 1. Organic acid washing solution preparation

Organic acid	Initial volume (ml)	Final volume (ml)
Acetic acid 1%	10	1000
Acetic acid 2%	20	1000
Acetic acid 4%	40	1000
Acetic acid 5%	50	1000
Organic acid	**Initial mass (g)**	**Final volume (ml)**
Citric acid 1%	10	1000
Citric acid 2%	20	1000
Citric acid 4%	40	1000
Citric acid 5%	50	1000

2.3. pH measurement

The pH levels of the washing solutions were measured and compared with the efficiency of the treatment solution. The pH of the treatment solutions was measured using a pH meter (Horiba D-75 mater, ABQ Industrial Inc., USA). The pH meter was calibrated before measurement.

2.4. Statistical analysis

The two t-test method was used for this study to determine the significant difference in treatment methods. A p-value of more than 0.05 is considered as not significantly different, and less than 0.05 is a statistically significant comparison between treatment and none treatment. In this experiment, we conducted two replications. Colony counts were converted into logarithmic values (log CFU/g), and means, and standard deviations were calculated.

3. Result and Discussion

3.1. Docker Image

3.1.1 Efficacy of organic acid washing solution

The average results for the viable bacteria count of various samples before washing are presented in this study. The mean of viable bacteria counts in unwashed (control) with all lettuce samples as control ranged from 6.14 log CFU/g to 6.57 log CFU/g. Based on a previous study, the mean viable bacteria count in fresh vegetables obtained in this study was similar to that of a recent study conducted, where analyzed lettuce showed a mean total variable bacteria count of 7.0 log CFU/g [18]. Likewise, aerobic plate counts can reach 9 log CFU/g, although most are in the 4 to 6 log CFU/g range, according to several studies [19, 20, 21]. The concentration of viable bacterial count in lettuce can be varied based upon different farm and hygiene practices throughout the production chain from harvesting, transportation, and storage.

As shown in Figure 1 and Figure 2, the result shows the variable bacteria reducing on lettuce after washing with different conditions of organic acid washing solution and deionized water. According to the result, the reduction of washing lettuce with deionized water in 10 min was 0.63 log CFU/g (10.19%). Based on the single acetic acid treatment, after 10 min of dipping washing with 1%, 2%, 4%, and 5% acetic acid (AA) solution, the reductions in viable bacterial count population were 1.95 log CFU/g (31.21%), 2.79 log CFU/g (44.58%), 3.60 log CFU/g (49.72%), and 3.70 log CFU/g (51.63%), respectively with significantly different ($p<0.05$). Organic acid, especially vinegar (acetic acid), generally recognized as a safe disinfectant by Food and Drug Administration (FDA) and the European Commission, are well accepted and consumers as an antimicrobial agent that are also considered to have great potential to control a wide range of microorganisms [22]. Similar to a previous study reported that the initial aerobic mesophilic microbial by > 3 log CFU/g of lettuce was reduced by acetic acid 2% and 4% (with a reduction of 3.37 and 3.91 log CFU/g, respectively) [23]. The number of viable bacteria count of treatment with 5% Acetic Acid (AA) is significantly different, which highest reducing bacterial loaded on lettuce samples. The best results were achieved with 5% acetic acid, which was significantly different ($P \leq 0.05$), and reduced the initial aerobic mesophilic population by soaking time for 10 minutes. Additionally, the pH level of 1%, 2%, 4%, and 5% acetic acid washing solution were 3.37, 3.31, 2.39, and 2.33, respectively.

Moreover, the result of viable bacteria counts on lettuces samples with single citric acid treatment, after 10 min of dipping washing with 1%, 2%, 4%, and 5% citric acid (CA) solution, the reductions in bacterial count population were 1.55 (26.80%), 1.73 (29.89%), 3.30 (46.05%) and 3.60 log CFU/g (49.18%), respectively. Among the four conditions of citric acid (CA) washing solutions, the concentration of 5% is a higher reduction than other conditions. With a high concentration of organic acid, our study illustrated the reduction of microbial higher than 3 log CFU/g. Furthermore, the 1, 2, 4, and 5% citric acid washing solution had pH of 3.2, 2.58, 1.94, and 1.88, respectively. The strongest acid solution in the study was the citric acid solution (pH = 1.88).

The result shows that a 5% acetic acid washing solution has the highest reduction compared to other organic acid washing solutions and deionized water. On the other hand, the pH level of acetic acid and citric acid assumed that lower pH could inhibit bacteria growth because this acidic washing solution killed the bacteria by breaking the bonds of nucleic acids and by precipitating proteins. The antimicrobial activity of organic acids is attributed to reduced pH by ionizing undissociated acid molecules. A low external pH can disrupt the substrate transport system by altering cell membrane permeability. In addition, citric and acetic acids have been researched in various food items for their antibacterial properties against microorganisms [24].

Table 2. Washing solution composition and level of pH

Organic acid washing solution		
Condition	**Composition**	**pH**
Deionized water	N/A	7.34
Acetic acid 1%	Acetic acid with deionized water	3.37
Acetic acid 2%	Acetic acid with deionized water	3.01
Acetic acid 4%	Acetic acid with deionized water	2.39
Acetic acid 5%	Acetic acid with deionized water	2.33
Citric acid 1%	Citric acid with deionized water	3.2
Citric acid 2%	Citric acid with deionized water	2.58
Citric acid 4%	Citric acid with deionized water	1.94
Citric acid 5%	Citric acid with deionized water	1.88
Commercial washing solution		
Deionized water	N/A	7.34
Commercial washing solution (A)	citric acid, sodium chloride, lemon flavor, and water	4.6
Commercial washing solution (B)	acid acetic, citric acid, sodium chloride, and water	3.6

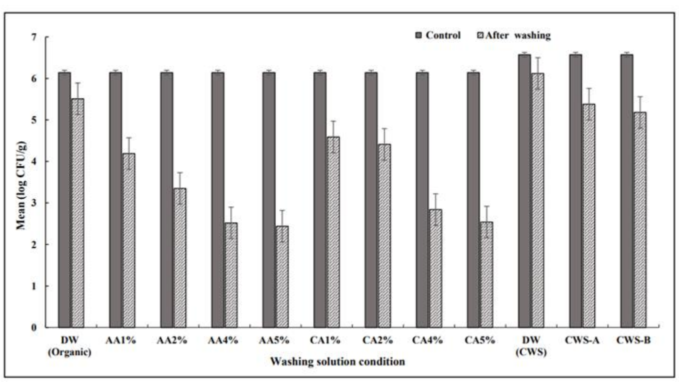

Fig. 1. The mean of viable bacterial count before (control) and after washing with organic acid solutions (log CFU/g)

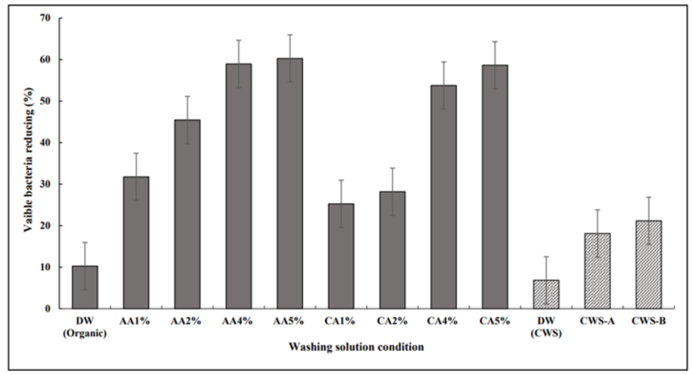

Fig. 2. The viable bacteria reduction after washing with organic acid solutions (%)

3.1.2. Efficacy of commercial washing solution

Before washing lettuce with different washing solutions such as commercial wash solutions A (CWS-A), commercial washing solution B (CWS-B), and Deionized water (DW), the viable bacteria count on the lettuce sample with no washing solution (control) was found to have 6.57 log CFU/g. One study mentioned that lettuce aerobic plate counts varied from 4.87 to 7.03 log10 CFU/g at a local market. It is generally agreed that microbial contamination of lettuce will arise if there is an improper operation in the process of its storage, transportation, shelf-life, handling equipment, etc. [25]. Similar results were reported from studies conducted in Spain and Brazil. In Spain, variable bacterial counts on lettuce samples at 16 university restaurants ranged from 3.01 to 7.81 log CFU/g [26]. While in Brazil, an analysis of ready-to-eat leafy salads revealed that 51% of them had counts of 6.0 log10 CFU/g [27].

Moreover, the result demonstrates that the variable bacteria count on lettuce after washing with CWS-A was 5.38 log CFU/g and CWS-B was 5.18 log CFU/g at 10min. In Figure 3 and Figure 4 show that, the level of bacterial reduction on lettuce at 10min was 0.45 log CFU/g (6.84%) for deionized water, 1.19 log CFU/g (18.11%) for CWS-A, and 1.39 log CFU/g (21.15%) for CWS-B. This result showed the CWS-B had more effective in reducing the microbial load in samples, which was statistically different ($p < 0.05$) from unwashed samples. Moreover, CWS-B was found to have a high ability to the reduction of bacterial count by comparison to CWS-A, this reason is due to CWS-B containing acetic acid 4%, citric acid 1%, and sodium chloride 3%, just different concentrations to inhibit the bacteria growth. These low-cost disinfectants are a new, eco-friendly way to keep the quality and safety of fresh foods intact. Similar to a previous study, the initial bacterial count by more than 1 log CFU/g of lettuce was reduced by acetic acid by 2%-4% with a reduction of 1 to 3 log CFU/g, respectively [29]. Conversely, it assumed that CWS-B could reduce the bacteria load because it has lower pH than CWS-A and DW. Also in this study, the washing solution such as CWS-A, CWS-B, and DW has pH levels 4.6, 3.6, and 7.34 respectively. Based on one study mentioned that lower pH of high acidity solution could have the ability to reduce microbial load on the vegetable sample [30]. On the other hand, the wash solution was an acid solution which could be the factor affect to bacteria growth due to pH, temperature, oxygen, and others and each of these factors influence the microbial population in a particular environment [31].

4. CONCLUSION

This study evaluated the efficiency of different organic acid solutions and commercial washing solution on removal of bacterial contamination the surface of vegetables In addition, among the different solutions, 5% acetic acid solution showed the highest microbial load reduction of tested vegetables, whereas washing with distilled water showed the lowest microbial reduction. In addition, the study also found that 'CWS-B' was the most effective antibacterial washing treatment to reduce microbial load on lettuce at a higher significance level than CWS-A wash solutions. Inhibition of microorganisms by organic acids depends upon several factors including reduction in pH, the ratio of undissociated species of the acid, chain length, cell physiology, and metabolism. The current study suggests that commercial antibacterial washing treatment should be updated to offer high effectiveness in eliminating dangerous bacteria from leafy vegetables. Further study should be conducting on formulation of washing solution that can remove not only viable bacteria but also pathogenic bacteria like Salmonella spp. or Escherichia coli.

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