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Survival of *Salmonella* in Diced Onions Washed by Different Concentrations of Pineapple Vinegar During Refrigerated Storage

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Abstract

Salmonella contamination has been repeatedly linked to diced onions. This study investigated the efficacy of washing solutions using pineapple vinegar at varying concentrations (0%, 5%, and 15%) on the survival of *S.* Typhimurium in diced onions during refrigerated storage conditions. Briefly, diced onions (50 g) were inoculated with *S.* Typhimurium at ~ 8 log CFU/g and then submerged in the washing solutions for 2 min. Inoculated onions were allowed to dry for approximately 1 hr, divided into 1 g portions and stored at refrigerated conditions for 12 days. *Salmonella* was enumerated on Xylose Lysine Deoxycholate (XLD) media at predetermined sampling points during storage. Overall, *S.* Typhimurium exhibited a little reduction (5.63 ± 0.34 log CFU/g) at 0% washing solution, whereas 6.25 ± 0.53 log CFU/g at 15% pineapple vinegar during the entire storage. *Salmonella* populations showed moderate reductions at 5% pineapple vinegar washing solution at the beginning of storage, but remained stable throughout the storage period. These findings suggest that pineapple vinegar could serve as a natural alternative to chemical preservatives, providing greater microbiological safety for diced onions while preserving their quality. The study highlights the role of natural antimicrobial agents in enhancing food safety and prolonging the shelf life of fresh produce.

INTRODUCTION

Food can be contaminated with *Salmonella* Typhimurium through water, soil, and surfaces. The risk of contamination is increased by improper handling and cross-contamination during post-harvesting, which includes processing and distribution [1,2]. The gastrointestinal symptoms of salmonellosis usually appear 12 to 72 h after consuming contaminated food. Children, the elderly, and people with impaired immune systems are among the vulnerable groups who face serious health hazards from these pathogens [3]. However, the risks of infection can be reduced by enforcing stringent hygiene regulations and vaccinating food handlers [4]. Therefore, effective prevention strategies include proper sanitation, food-handling practices, and novel decontamination methods. *Salmonella* is known for its adaptability to a variety of environmental conditions due to its gram-negative, facultatively anaerobic nature. Recently, fresh produce was linked to *Salmonella* Typhimurium contamination [5-7], a major foodborne illness-causing serotype usually associated with poultry and eggs [8]. For instance, in 2024, outbreaks throughout the United States were linked to fresh basil and cucumbers [6,7]. In addition to that, fresh papayas (in 2019), alfalfa sprouts (in 2022), and cantaloupes (in 2023) were the subjects of other outbreaks [5-7,9]. On that account, the frequency of these outbreaks emphasizes the need for different fresh product washing techniques.

Although onions have a minimal correlation with *Salmonella*, they are susceptible to microbial contamination, as are other fresh vegetables. For instance, *Salmonella* Newport (in 2020) and *Salmonella* Oranienburg (in 2021) are two of the most notable outbreaks associated with onions documented [10]. The source of contamination varies from cross-contamination during food preparation the processing equipment [11]. As a result, food

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safety procedures are made more difficult by the bacterium's high flexibility in response to environmental factors, such as changing temperatures and moisture levels [12]. Therefore, these *Salmonella*-related foodborne outbreaks emphasize the urgent need for improved intervention methods.

Pineapple vinegar is rich in organic acids, such as acetic, citric, and malic acids, which are known for their antimicrobial properties, and is produced from fermented pineapple pulp and skin. The presence of bromelain and a proteolytic enzyme can enhance its antibacterial efficacy [13,14]. According to studies, vinegar is a good natural food preservative because it effectively reduces microbial loads on food surfaces [15]. The antimicrobial potential of pineapple vinegar is due to acetic acid, which damages bacterial cell membranes, and flavonoids, which possess anti-inflammatory and antiviral activity [1]. Various studies have since investigated vinegar's ability to reduce microbial loads in food products, thereby advocating for its application as a safer alternative to chemical preservatives [16]. Besides, due to the advantageous probiotic properties of pineapple vinegar, gut health is stimulated, a factor that further promotes its use as a natural food additive [17].

Since prehistoric times, vinegar has been used for food preservation, especially pickling. Pineapple vinegar is being investigated for its efficacy in reducing bacterial contamination of meat and fresh produce in current food safety applications [2]. It has an acidic characteristic, helping extend the shelf life of food by inhibiting bacterial growth and reducing reliance on synthetic preservatives [13]. Optimizing the application of pineapple vinegar in food processing to ensure microbial safety and preserve sensory characteristics is a focus of recent studies [18]. In light of increasing concerns regarding *Salmonella* contamination of fresh produce, this study aimed to evaluate the efficiency of pineapple vinegar in reducing *Salmonella* Typhimurium in diced onions. By investigating a natural, sustainable antimicrobial agent, this study helps advance food safety and aligns with consumer preferences for using as few chemical additives as possible. The findings could further affect industry practice, regulations, and research into alternative food-preservation techniques.

MATERIALS AND METHODS

Materials

Fresh red onions (*Allium cepa*) (2 kg) were obtained from a local market. The red onions were selected for uniformity in size and absence of visual defects. Red onions were stored at 7-12 °C to maintain freshness [19]. Pineapple vinegar (3.55 pH) was obtained from Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA). It was diluted into two concentrations (5% and 15%) in sterile distilled water, each prepared in a total volume of 500 mL, and used as washing solutions.

Inoculum preparation

Stock culture of *Salmonella* Typhimurium was revived in Tryptone Soy Broth Yeast Extract (TSBYE) and incubated at 37 °C for 18-24 h. The culture was streaked onto Tryptone Soy Agar Yeast Extract (TSAYE) plates to obtain single colonies, which were maintained at 4 °C and subcultured biweekly. A single colony was transferred to fresh TSBYE broth and incubated at 37 °C for 24 h. The overnight culture was spread on TSAYE plates and incubated at 37 °C for another 24 h. The bacterial lawn was harvested using 0.1% buffered peptone water (BPW) and serially diluted to achieve a final concentration of 8 log CFU/mL.

Background microflora test

To determine the initial microbial load, red onions were diced into 1 cm × 1 cm × 1 cm cubes. A 25 g sample was homogenized in 225 mL of 0.1% BPW, followed by serial dilution (10^0, 10^{-1}, 10^{-2}). Aliquots were plated on XLD agar (*Salmonella*), Potato Dextrose Agar (yeast/mould), and Plate Count Agar (PCA) and incubated under appropriate conditions.

Preparation and inoculation of diced onions

Fresh onions were peeled and diced into 1 cm × 1 cm × 1 cm cubes. A 50-g portions were inoculated with 0.5 mL of *S.* Typhimurium to achieve ~8 log CFU/g. The mixture was hand-massaged for 3 min and spread on aluminum foil to dry under a biosafety hood.

Preparation of pineapple vinegar washing solutions

Three washing solutions were prepared: WS1 (5%) and WS3 (15%) pineapple vinegar, as per the calculations shown in **Table 1**. Each formulation was mixed in a sterile Schott bottle. Diced onions were submerged in these solutions for 2 min and collected using a sterile strainer.

Table 1. The formulations of washing solutions for treatment preparation.

Washing solutions (%)	Amount of pineapple vinegar (mL)	Amount of sterile distilled water (mL)
0	0	500
5	25	475
15	75	425

Refrigerated Storage of Treated Diced Onions

Inoculated and treated onions were stored at 4-7 °C for 12 days. Samples were collected at intervals (0 h, 2 h, days 1-5, 8, 10, and 12) for analysis.

Enumeration of *Salmonella* During Refrigerated Storage

Two replicate samples were homogenized in 9 mL of 0.1% BPW and serially diluted (10^{-1}, 10^{-2}, 10^{-3}). A 0.1 mL aliquot was plated on XLD agar, incubated at 37 °C for 24 h, and black colonies counted. Colony counts were expressed as log CFU/g using the equation:

$$CFU/g = \frac{Number\ of\ colony\ counts \times Dilution\ factor}{Aliquot}$$

Microbial Analysis and Data Interpretation

Microbial counts were statistically analyzed to assess the efficacy of pineapple vinegar in reducing *S.* Typhimurium contamination. The reduction trends of *S.* Typhimurium were evaluated across different washing solutions and refrigerated storage conditions. The results were interpreted to determine pineapple vinegar's effectiveness as a natural antimicrobial agent for controlling *Salmonella* in diced onions. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$) using Microsoft Excel.

RESULT AND DISCUSSION

Background microbial counts

Fresh vegetables, including onions, naturally harbor microorganisms, making background microbial analysis crucial before *Salmonella* inoculation. In this study, each new batch of onions underwent microbial testing to assess the presence of yeast, mould, viable bacteria, and *Salmonella*. Across five onion batches, yeast and mould counts on PDA plate consistently remained below 25 CFU/g, while no viable bacteria or

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Salmonella were detected on PCA and XLD agar (**Table 2**). These results highlight the onions' natural antimicrobial properties, aligning with findings by Ahmed et al. (2016), who reported that onion extract at 6.5 mg/mL completely inhibited the growth of *Salmonella*, E. *coli*, and *Bacillus subtilis*. The absence of viable bacteria and *Salmonella* in the initial Total Plate Count (TPC) confirms the onions' antibacterial activity, while minimal yeast and mould levels ensured that *Salmonella* inoculation would not be compromised by competing background microflora. This is consistent with a study that found that excessive background microflora can inhibit the growth of target pathogens such as *Salmonella* [20].

Although onions may carry fungi such as *Aspergillus niger* (black mould), *Fusarium* (basal rot), and *Botrytis* (neck rot), these risks are minimized through proper sample preparation and storage [21,22]. The consistent suppression of microbial growth indicates that the onions used were suitable and safe for experimental purposes. *Salmonella* Typhimurium detection was performed using XLD agar, a selective medium ideal for isolating enteric pathogens. Its formulation, including sodium deoxycholate, inhibits non-enteric bacteria, while allowing *Salmonella* to grow as red colonies with black centers due to hydrogen sulfide (H_2S) production from xylose fermentation and lysine decarboxylation [23,24]. The absence of such colonies in uninoculated samples confirmed that the onions were free from background *Salmonella* contamination. In conclusion, the results from **Table 2** affirm the antimicrobial reliability of the onion samples used in this study. The consistently low microbial counts and the absence of *Salmonella* validate the suitability of the onions for controlled experimental conditions, supporting previous research on their antibacterial properties [25].

Table 2. Background microflora counts in onions by batch.

Batch	Total Plate Count (CFU)	Yeast and Mould Count (CFU)	*Salmonella* Typhimurium Presence (CFU)
1	not detected (nd)	< 25	nd
2	nd	< 25	nd
3	nd	< 25	nd
4	nd	< 25	nd
5	nd	< 25	nd

Homogeneity test

After inoculating *Salmonella* into diced onion samples, a homogeneity test was performed on each batch to ensure even bacterial distribution. This test verified that the observed effects were due to the washing solutions, not to external variables such as onion type, starting microbial load, or storage conditions. Variability in *Salmonella* survival between treatments is shown in **Table 3**. The control (0% washing solution) had an average *Salmonella* population of 5.21 ± 0.53 log CFU/g. The 5% washing solution reduced the population to 4.15 ± 0.00 log CFU/g, indicating its effectiveness, while the 15% solution reduced the population to 3.91 ± 0.75 log CFU/g, demonstrating similar reduction levels at higher concentrations. The variability suggests slight inconsistencies in effectiveness with higher concentrations, as reflected in the standard deviations. The variability in initial population counts is likely due to the washing process, during which *Salmonella* is removed by submersion in the washing solutions. Some *Salmonella* may have been lost during washing due to physical removal from the onion surfaces. The rate of agitation during submersion could also contribute to population variability.

Additionally, the natural antimicrobial agents in diced onions may have influenced *Salmonella* reduction, as their concentration can vary across onion batches due to external factors, thereby affecting the observed reduction [26].

Table 3. Average of *Salmonella* Typhimurium population (log CFU/g) in diced onions after washing as affected by pineapple vinegar concentrations (n=2) at 0 h.

Washing solutions with different concentrations of pineapple vinegar (%)	Average of *Salmonella* population after washing at 0 h (log CFU/g)
0	5.21±0.53[a]
5	4.15± 0.00[a]
15	3.91±0.75[a]

Note: Same letters within the same column indicate there is no significant differences (p>0.05) between mean values.

Effectiveness of Treatments on the Reduction of *Salmonella* Population from First Washing

The effectiveness of various washing solutions in reducing *Salmonella* populations on diced onions was evaluated, as shown in **Table 4**. The control treatment (0% washing solution) reduced the *Salmonella* population by 5.60 ± 0.53 log CFU/g, indicating that washing with water alone has limited effectiveness in reducing *Salmonella* levels. A study noted that washing without chemical agents does not significantly reduce harmful bacteria [27]. While the absence of antimicrobial chemicals limited chemical inactivation, the physical washing process likely removed some surface-attached bacteria. In addition, a study suggested that onions' inherent antibacterial properties may have contributed to the observed decrease in *Salmonella* populations [28].

The 5% washing solution reduced *Salmonella* by 6.65 ± 0.00 log CFU/g, showing a higher reduction than the control. A study attributed this result to the antimicrobial compounds in pineapple vinegar, which effectively suppress bacterial populations even at lower concentrations [14]. However, the reduction was still modest and may not meet microbiological safety standards for high-risk foods. The 15% washing solution showed the most significant reduction in *Salmonella*, reducing the population by 6.89 ± 0.75 log CFU/g. This solution's higher concentration of pineapple vinegar significantly enhanced its antimicrobial activity, as organic acids (e.g., acetic acid) damage bacterial cell membranes, hinder metabolic processes, and lower the environment's pH, leading to bacterial cell death [14, 29]. Despite *Salmonella*'s moderate resistance to acid, the acidic environment of the washing solution was particularly effective in reducing bacterial growth [30]. The 15% solution's high concentration of antibacterial chemicals ensures effective decontamination of diced onions.

Compared with the control, the 15% washing solution demonstrated a significantly greater decontamination effect, making it suitable for high-risk foods. In contrast, the 5% washing solution resulted in mild reductions, which may not be sufficient to control *Salmonella* growth. The addition of washing solutions provided notable benefits over the control, emphasizing the importance of antimicrobial agents for effective bacterial control. However, variations in bacterial load and antimicrobial properties of the onions, influenced by factors such as different purchase days, handling procedures, and antimicrobial agent content in the onions, may also contribute to the observed variability in results [26,31].

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Table 4. Reduction of *Salmonella* Typhimurium population (log CFU/g) in diced onions after washing as affected by pineapple vinegar concentrations (n=2).

Washing solutions with different concentrations of pineapple vinegar (%)	Reduction of *Salmonella* after washing (log CFU/g)
0	5.60 ± 0.53[a]
5	6.65 ± 0.00[a]
15	6.89 ± 0.75[a]

Note: Same letters within the same column indicate there is no significant differences (p>0.05) between mean values.

Effectiveness of Pineapple vinegar in Inhibiting *Salmonella* Typhimurium Growth in Diced Onions

The effectiveness of Pineapple vinegar by different concentrations in inhibiting *Salmonella* Typhimurium growth in diced onions during refrigerated storage was as shown in **Fig. 1**.

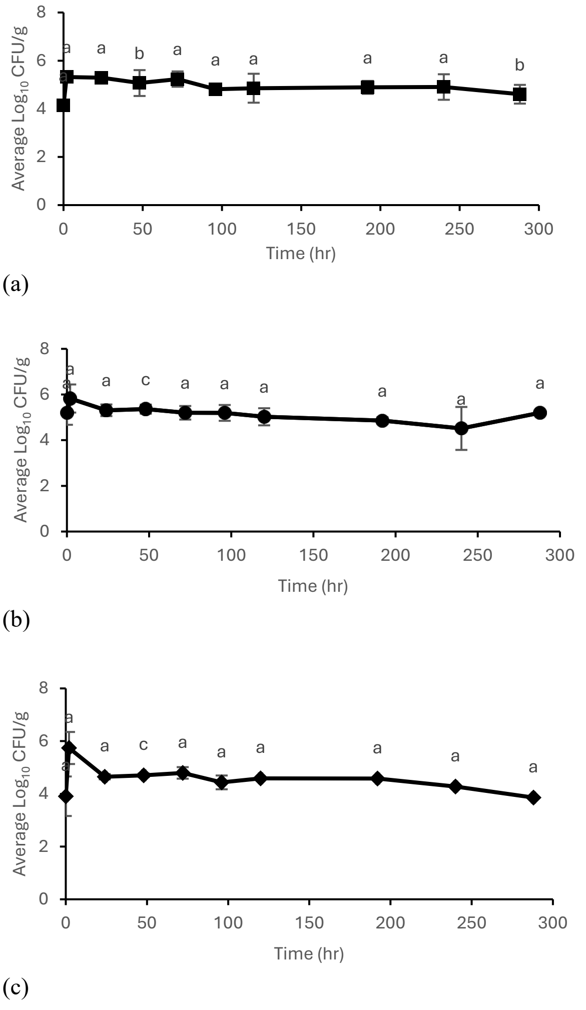

Fig. 1. *Salmonella* Typhimurium population in diced onions during refrigerated storage of 12 days in different washing solutions as affected by a) control; b) 5%; c) 15%. For the washing solution treatments (0%, 5%, and 15%), Tukey's post-hoc significance groupings were applied for every storage time point. A one-way ANOVA combined with Tukey's HSD test shows that treatments that have the same letter are not significantly different ($p > 0.05$).

Table 5. The statistical test and p-value for different washing solutions (0%, 5% and 15%) over the 12-day storage period.

Washing solutions (%)	F-statistic	p-value
0	1.493	0.270
5	1.423	0.295
15	4.561	0.013

Based on **Table 5**, 0% and 5% washing solutions showed there is no significant difference for *Salmonella* reduction over time (p>0.05), which contradicts the 15% washing solutions, where the treatment indicated that the 15% pineapple vinegar had a statistically significant impact on the reduction of the *Salmonella* population over the storage period.

Diced onions with 0% washing solutions during refrigerated storage

In the control group using 0% washing solution (sterile distilled water), *Salmonella* Typhimurium populations showed only minimal reduction during refrigerated storage. Initial reductions observed at 0 h (before refrigeration) were attributed to the physical removal of surface-attached bacteria through rinsing. However, this effect was temporary. Over time, *Salmonella* levels remained relatively stable and even regrew after 10 days of storage at 4-7 °C. This outcome indicates that while washing with water may dislodge some microbes, it lacks antimicrobial efficacy, and refrigeration alone is insufficient for controlling *Salmonella* contamination [32]. The persistent and eventual increase in bacterial population suggests *Salmonella*'s ability to adapt to cold stress and maintain metabolic functions, allowing it to survive and possibly proliferate under refrigerated conditions [33].

The regrowth observed after prolonged storage also underscores *Salmonella*'s resilience in the absence of antimicrobial treatments. This aligns with findings that *Salmonella* can resist cold environments by altering gene expression to support survival [32]. Furthermore, diced onions themselves may provide a supportive environment for pathogen survival, offering moisture and nutrients that facilitate bacterial persistence [26]. Overall, these findings emphasize the limitations of relying solely on refrigeration and water rinsing for microbial safety in fresh-cut produce. Without the inclusion of an antimicrobial agent, *Salmonella* remains viable and can rebound over time. This underscores the need for additional decontamination strategies, such as chemical sanitizers or natural antimicrobial solutions, to reduce microbial loads more effectively and enhance food safety.

Diced onions with 5% washing solutions during refrigerated storage

The application of 5% pineapple vinegar as a washing solution resulted in an initial reduction of *Salmonella* Typhimurium populations on diced onions during refrigerated storage. This early antimicrobial effect is likely due to the acidity and bioactive compounds in pineapple vinegar, which can lower pH and disrupt bacterial activity [34]. Alongside the physical removal of bacteria during the initial wash, the vinegar's natural antimicrobial properties contributed to the initial drop in *Salmonella* counts. However, this reduction was not maintained throughout the storage period. After the initial decline, *Salmonella* levels stabilized rather than continuing to decline, suggesting that the antimicrobial effect of the 5% solution was insufficient to sustain inhibition over time.

The limited efficacy may be due to the relatively low concentration of antimicrobial compounds, which, when diluted to 5%, may not have been potent enough to continue suppressing bacterial growth during storage [34]. Several factors may have contributed to this outcome. First, the antimicrobial components in vinegar, such as acetic acid and phenolics, may have been absorbed or neutralized upon contact with the diced onions, reducing their availability over time. Second, a study found that antimicrobial agents often exhibit concentration-dependent effectiveness; lower concentrations may not be sufficient to eliminate resilient pathogens such as *Salmonella* [35]. Additionally, *Salmonella* possesses adaptive mechanisms that allow it to survive in acidic environments, which could diminish the long-term effectiveness of mild acidic treatments like 5% pineapple vinegar [36]. In summary, while a 5% pineapple vinegar washing solution demonstrated initial antibacterial activity against *Salmonella* Typhimurium, its low concentration was insufficient to sustain inhibition throughout refrigerated storage. This emphasizes the importance of optimizing antimicrobial concentration in natural sanitizers to ensure long-term microbial safety in fresh-cut produce.

Diced Onions with 15% Washing Solution During Refrigerated Storage

Among all tested concentrations, the 15% pineapple vinegar washing solution demonstrated the most significant and sustained reduction of *Salmonella* Typhimurium populations on diced onions during refrigerated storage. This suggests a strong and lasting antimicrobial effect, attributed to the higher concentration of bioactive compounds present in the pineapple vinegar solution [14]. The enhanced efficacy of the 15% solution is likely due to elevated levels of phenolic compounds and natural antioxidants in pineapple vinegar. These compounds are known for their broad-spectrum biological activity, including antimicrobial effects, particularly when present in higher concentrations [37]. The increased concentration intensifies oxidative stress on bacterial cells, leading to the breakdown of essential components such as proteins, lipids, and DNA. This damage disrupts cell integrity and function, effectively inhibiting bacterial growth and survival [38].

The success of the 15% treatment suggests that the combined effects of oxidative damage and antimicrobial pressure from pineapple vinegar were strong enough to prevent not only *Salmonella*'s initial survival but also its regrowth during prolonged cold storage. This contrasts with the lower concentrations, where bacteria either adapted or survived due to insufficient antimicrobial stress. Moreover, cold storage (4-7 °C) itself contributes to limiting bacterial growth, though it is not bactericidal [39]. When combined with a potent washing solution like the 15% pineapple vinegar, refrigeration can help create an inhospitable environment for *Salmonella*, reducing its ability to recover or multiply.

The dual effects of strong oxidative stress from pineapple vinegar and metabolic inhibition from refrigeration likely explain the pronounced bacterial reduction observed in this treatment group. In conclusion, the 15% pineapple vinegar washing solution was the most effective concentration tested, offering substantial antimicrobial activity throughout storage. These results highlight the potential of higher-concentration natural sanitizers in improving the microbial safety of fresh-cut produce. The effectiveness of pineapple vinegar in inhibiting *Salmonella* Typhimurium growth in diced onions during storage was as reported in **Table 6**.

Table 6. Reduction of *Salmonella* Typhimurium population (log CFU/g) in diced onions after storage as affected by pineapple vinegar concentrations (n=2).

Washing solutions (%)	Overall reduction of *Salmonella* population after storage (log CFU/g)	Reduction of *Salmonella* population at day 12 of storage (log CFU/g)
0	5.63 ± 0.37^{b}	5.60 ± 0.00^{b}
5	5.89 ± 0.31^{b}	6.20 ± 0.39^{a}
15	6.25 ± 0.03^{a}	6.94 ± 0.00^{a}

The storage duration was 12 days for solutions with 0%, 5%, and 15% pineapple vinegar. Different letters within the same column indicate there is significant differences ($p<0.05$) between mean values. This study investigated the survival of *Salmonella* Typhimurium in diced onions treated with pineapple vinegar at varying concentrations during refrigeration (4-7 °C). The antimicrobial effectiveness of pineapple vinegar was assessed by comparing the washing solutions (5% and 15%) to a control (0%). A 12-day storage period was used to observe *Salmonella* reduction. Results showed a low initial microbial load, confirmed by background microflora testing. The 15% pineapple vinegar solution was the most effective, significantly reducing *Salmonella* populations throughout storage. Lower concentration (5%) showed limited and inconsistent reductions, likely due to insufficient active compounds in the vinegar. *Salmonella* populations in the control group increased over time, indicating that refrigeration alone (0% solution) was ineffective. These findings suggest that high-concentration pineapple vinegar can enhance the microbiological safety of diced onions during refrigerated storage.

CONCLUSION

This study confirmed that pineapple vinegar is an effective natural antibacterial agent against *Salmonella* Typhimurium in diced onions, particularly at higher concentrations. The 15% concentration showed a significant reduction in *Salmonella* populations and maintained lower levels throughout refrigerated storage. Lower concentrations (5%) provided moderate reductions, but the effects were not sustained over time. The control group showed that physical washing or chilling alone could not control *Salmonella* growth. These results highlight the importance of incorporating antimicrobial treatments, such as pineapple vinegar, into food safety practices for ready-to-eat vegetables to combat foodborne pathogens.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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