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Effectiveness of Varying Concentrations of Salt and Vinegar against the Bacterial Contaminants of Fruits and Vegetables

Yuguda U. H.[1,2], Abdullahi M. M.[2], [2]Isa S. and Garba L.[2]*

[1]Department of Biological Sciences, Faculty of Science, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria

[2]Department of Microbiology, Faculty of Science, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria

Correspondence Authoremail: lagarpak@gsu.edu.ng

ABSTRACT

Fruits and vegetables, which are of human diets, are regularly tainted by various microbes. Microbial contamination can arise from various causes such as environment, animals, human activity, through transportation or preparation of produce from the farm to the table. The purpose of this study was to evaluate the effects of different vinegar and salt concentrations on the bacterial loads of several fruits and vegetables that are sold in the city of Gombe. For the evaluation, a total of thirty fruits and vegetables from various suppliers were used. A portion of each sample was subjected to a ten-fold serial dilution prior to treatment with either of the two agents. Aliquots of the 10^{-1}, 10^{-2}, and 10^{-3} dilutions were then inoculated using the pour-plate technique on nutrient agar and MacConkey agar plates. The plates were incubated at 37°C for 24 hours during which the counts of aerobic mesophilic and faecal coliform were observed. Results of the bacterial loads observed prior to washing ranged from $3.7x10^4$ to $2.7x10^5$ and $2.9x10^4$ to $8.7x10^4$ for total mesophilic and faecal coliform count, respectively; however, a significant decrease of the bacterial loads of up to 0.77-1.91 and 0.50-1.23 log was observed following treatment with vinegar and salt solutions, respectively. It was concluded that vinegar was significantly more effective than salt in reducing the bacterial loads of the fruits and vegetables ($P<0.05$). In summary, the fruits and vegetables were found to be contaminated with high bacterial loads that decreased with increased concentrations as well as the exposure time of the vinegar and salt solutions.

Keywords: Fruits, vegetables, contamination, vinegar, salt, effectiveness

INTRODUCTION

According to Abadias *et al*. (2008) and the WHO/FAO (2008), fresh fruits and vegetables are a vital component of a human diet since they provide sources of proteins, carbohydrates, and several vitamins and minerals that are necessary for a healthy life. Consumers have been urged to eat more fruits and vegetables because of their widely acknowledged importance in wholesome diets (WHO, 2012). Actually, consuming them not only slows the onset of illness, but their fibres also regulate how the body breaks down food (de Moura *et al*., 2014).

Different types of microorganisms can contaminate fresh produce (Berger *et al*., 2010). Fruits and vegetables are prone to microbial contamination due to contact with soil, dust, and water, as well as handling during harvest or postharvest processing. Consequently, they harbour a diverse range of microorganisms including plant and human pathogens (Carmo *et al*., 2004; Verhoeff-Bakkenes *et al*., 2011). Unrelated factors like resident micro flora in the soil, introduction of non-resident micro flora via animal manures, sewage or irrigation water, transportation, and handling by retailers are largely responsible for differences in microbial profiles of different fruits and vegetables (Ray and Bhunia, 2007; Ofor *et al*., 2009). One of the main causes of contaminations in developing nations like

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Nigeria is the ongoing use of manure and untreated waste water as fertilizers for the growth of fruits and vegetables (Amoah *et al*., 2009).

According to some studies (Abadias *et al*., 2008; Aycicek *et al*., 2006;Verhoeff-Bakkenes *et al*., 2011), fresh produce may carry enterotoxigenic and enterohemorrhagic *Escherichia coli*, Salmonella spp., *Listeria monocytogenes*, and thermotolerant Campylobacter spp., as well as parasitic and viral pathogens that can infect humans. Other studies have demonstrated the presence of possible food-borne pathogens in fresh fruits and vegetables (Beuchat, 2002; Warriner *et al*., 2009). Large-scale foodborne outbreaks around the world are caused by pathogenic *E. coli* and Salmonella spp., which can cause persistent infections and gastrointestinal symptoms. Ready-to-eat veggies can become contaminated by the psychrotolerant, common bacteria *L. monocytogenes*, which also causes listeriosis (De Oliveira *et al*., 2011). Ready-to-eat items have been shown to include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Bacillus spp., Proteus spp., Lactobacillus spp., and Klebsiella spp, (Oranusi and Olorunfemi, 2011).

The process of washing vegetables during their manufacturing has been found to be a possible vector for the dispersal of germs, particularly *E. coli*, into the final product. In addition to washing vegetables with potable water, sanitizers have been used to further disinfect the produce, helping to get rid of bacteria to some extent. However, fresh-cut produce has a limited capacity to eliminate naturally occurring microorganisms (0.5–2.0-log reduction); in other words, some microbial reduction happens but entire reduction is not possible. These restrictions are ascribed to surface irregularities or microbial adhesion, particularly at cut edges or cracks in surfaces (Holvoet *et al*., 2012; Van Haute *et al*., 2013; WHO, 2015).

Both physical and chemical treatment procedures have been thoroughly researched for decontamination of fresh-cut fruit and vegetables (Rahman *et al*., 2021). The elimination of microbial burdens from fresh fruits and vegetables is considerably enhanced by disinfectant wash. While a number of antimicrobial agents have been shown to be effective against bacterial contaminants found in fruits, vegetables, and other sources (Addo *et al*., 2020; Rahman *et al*., 2021; Baldas & Altuner, 2018; Garba *et al*., 2019), the majority of these agents are not suitable for use in household treatments. For example, the initial stage of processing for ready-to-eat salads is washing with free chlorine, which is mostly utilized in commercial methods to reduce microbial contamination.

Unfortunately, a growing number of customers are abstaining from eating goods that have been chemically preserved. Thus, it is crucial to evaluate alternative options, especially natural products, for sanitizing fresh fruits and vegetables (Addo *et al*., 2020). Accordingly, Atter *et al*. (2014) evaluated the effectiveness of Water, Vinegar and Salt in reducing bacterial population of fresh green cabbage in Ghana. Their study revealed that vinegar was more effective in reducing the bacterial loads than the salt and plain tap water. Another report from Ghana by Addo *et al*. (2020) showed that vinegar was the most effective in reducing the microbial load of lettuce when compared with salt and lemon juice. A Similar report by Rahman *et al*. (2021) from Bangladesh confirmed the efficacy assessment of some low-cost disinfectants including salt water, blanched, vinegar and sterile water in reducing the microbial loads of selected fruits and vegetables. Even though vinegar was found to be the most effective, all treatments were significantly effective in reducing the fruits and vegetables contaminations.

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These cited reports affirmed that many studies revealed the effectiveness of natural disinfectants in reducing microbial loads of fruits and vegetables. However, the paucity of scientific proof on the effectiveness of salt and vinegar solutions used by the host community in the treatments of fruits and vegetables makes this research very relevant. Therefore, the purpose of this study was to determine the effectiveness of varying concentrations of vinegar and salt in reducing the bacterial loads of fruits and vegetables sold in Gombe metropolis, Gombe State, Nigeria as the first report on the effect of natural disinfectants against the bacterial contaminants of both fruits and vegetables to the best of our knowledge.

MATERIALS AND METHODS

Sample Collection

Thirty (30) samples, five (5) of each of the following: water melon (*Citrullus lanatus*), guava (Psidium guajava), apple (*Malus domestica*), cabbage (*Brassica oleracea*), carrot (*Daucus carota*), and lettuce (*Lactuca sativa*), were acquired from several vendors at the Gombe main market. The samples were placed in sterile polythene bags and taken right away to the laboratory for analysis.

Determination of Bacterial Loads of the Samples

Hundred millitre (100 ml) of sterile distilled water was used to wash twenty-five grams (25g) of fruits or vegetables. Ten-fold serial dilution of the rinsed water was made, and an aliquot of 0.1 ml of the 10^{-1}, 10^{-2}, and 10^{-3} dilutions was pour plated on nutrient agar and MacCkonkey agar for aerobic mesophilic and feacal coliform counts, respectively. Following an 18–24 hour incubation period at 37^0C, the colonies form on the inoculated plates were enumerated and expressed as colony forming units (cfu/g) per gram of sample (Clarence *et al*., 2009).

Determining the Impact of Salt and Vinegar Solutions on the Samples' Bacterial Loads

In sterile distilled water, vinegar solutions at different concentrations of 15, 20, and 25% were made in order to assess the impact of the solution on the bacterial burdens of fruits and vegetables. Twenty-five grams of the fruits or vegetables were washed with each of the concentrations. On plate count agar, an aliquot containing 0.1 ml of each concentration was inoculated at the zero minute, five minutes, and ten minutes of exposure. Number of colonies on each plate was counted using a colony counter following an overnight incubation at 37 ^{o}C (Eni *et al*., 2010). In a similar manner, 100 ml of 15, 20, and 25% salt solutions made with sterile distilled water were used to wash 25g of fruit and vegetable samples each. Each rinsed solution was inoculated with an aliquot containing 0.1 ml on a plate count agar at 0 minutes, then exposed for 5 and 10 minutes before being incubated for 24 hours at 37 ^{o}C. A colony counter was used to count the number of colonies on each plate (Eni *et al*., 2010).

Statistical Analysis

The results were analysed using One-way ANOVA in Microsoft Excel 2023 version.

RESULTS

Before evaluating the efficacy of the vinegar or salt solutions, the bacterial loads of both products were ascertained, as indicated in Table 1. The outcome demonstrated a broad spectrum of bacterial contamination ranging from 3.8×10^4 to 1.1×10^5 of total viable counts as published in our previous work (Garba *et al*., 2024).

Effectiveness of Vinegar Solution in Reducing the Bacterial Loads of Fruits and Vegetables

The total bacterial loads of all the fruits and vegetables exposed to different concentrations of vinegar (15, 20, and 25%)

at different time intervals (0, 5, and 10 minutes) are displayed in Figure 1. At 15% vinegar concentration and initial exposure time (zero min), the least amount of bacterial load (highest reduction) was observed in guava (3.76 log CFU/g), followed by apple (3.81 log CFU/g), each of cabbage and lettuce (3.89 log CFU/g), water melon (3.93 log CFU/g), and carrot (3.94 log CFU/g). Overall, a reduction of bacterial load was observed among all the fruits and vegetables after every increase in vinegar concentrations and exposure time.

Table 1: Viable bacterial counts of fruits and vegetables.

S/N	Fruit/vegetable	VBC (CFU/g)	Log10
1	Apple	3.8×10^4	4.58
2	Guava	3.7×10^4	4.57
3	Water melon	1.1×10^5	5.04
4	Cabbage	1.1×10^5	5.04
5	Carrot	2.7×10^5	5.43
6	Lettuce	1.7×10^5	5.23

Key: VBC: Viable bacterial count, CFU=colony forming unit, g=gram

At the beginning of contact time, the guava had the biggest reduction (3.54), followed by the apple (3.62 log CFU/g), lettuce (3.68 log CFU/g), water melon (3.72 log CFU/g), cabbage (3.76 log CFU/g), and carrot (3.83 CFU/g). Guava (3.56 log CFU/g) showed the largest reduction at 20% vinegar concentration in the beginning, followed by apples (3.64 log CFU/g), water melon (3.75 log CFU/g), cabbage (3.77 log CFU/g), and carrots (3.84 log CFU/g).

The reduction in bacteria was greatest in guava (3.34 log CFU/g) after 10 minutes of contact. This was followed by lettuce (3.56 log CFU/g), water melon (3.58 log CFU/g), cabbage (3.62 log CFU/g), apple (3.43 log CFU/g), and carrot (log CFU/g 3.70). Guava had the largest reduction in bacterial load at the beginning time (3.43 CFU/g) at a 25% vinegar concentration, followed by apples (3.51 CFU/g), water melons (3.54 CFU/g), lettuce (3.58 CFU/g), cabbage (3.59 CFU/g), and carrots (3.70 CFU/g). After a 10 minute exposure period, guava (2.95 CFU/g) showed the greatest reduction, which was followed by carrot (3.56 CFU/g), apple (2.97 CFU/g), water melon (3.30 CFU/g), lettuce (3.32 CFU/g), and cabbage (3.38 CFU/g).

Effectiveness of Salt Solution in Reducing the Bacterial Loads of Fruits and Vegetables

Figure 1 displays the final bacterial load following treatment with salt solutions. Similar to vinegar, the bacterial population was decreased by the salt solution as exposure time and concentration increased from 15–25%. Guava showed the largest reduction in bacterial load (3.89 CFU/g) at 15% salt content and starting time, followed by apples (4.08 CFU/g), water melons (4.11 CFU/g), carrots (4.20 CFU/g), cabbage (4.23 CFU/g), and lettuce (4.26 CFU/g). The results indicate that after a 10 minute exposure time, guava had the biggest reduction (3.72 CFU/g), followed by lettuce (4.15 CFU/g), apple (3.94 CFU/g), water melon (3.99 CFU/g), carrot (4.08 CFU/g), and cabbage (4.11 CFU/g).

The first time reduction in CFU/g was largest in guava (3.76 CFU/g) at 20% salt concentration, followed by apple (3.95 CFU/g), water melon (4.00), carrot (4.08), lettuce (4.15), and cabbage (4.18 CFU/g). After a 10-minute exposure period, guava showed the largest reduction (3.60), which was followed by lettuce (4.08 CFU/g), apple (3.79 CFU/g), water melon (3.89 CFU/g), carrot (3.99 CFU/g), and cabbage (4.04

CFU/g). The greatest reduction in the beginning was observed in guava (3.60 CFU/g) at a 25% salt concentration, followed by carrot (3.73 CFU/g), apple (3.82 CFU/g), water melon (3.90 CFU/g), cabbage (4.04 CFU/g), and lettuce (4.08 CFU/g). Guava (3.51 CFU/g) showed the largest reduction after 10 minutes of exposure, followed by apples (3.64 CFU/g), water melon (3.73 CFU/g), carrots (3.84 CFU/g), cabbage (3.89 CFU/g), and lettuce (4.00 CFU/g).

DISCUSSION

Fruits and vegetables are frequently contaminated by microorganisms at every stage of the farm-to-table process, environmental factors, animal sources, and human activity. A wide variety of microorganisms, such as plant and human pathogens, are present in them (Carmo *et al*., 2004; Eni *et al*., 2010; Garba *et al*., 2024). Since fruits and vegetables are frequently eaten raw without being further heated, there is always a chance of contracting food poisoning or another food-borne illness (Aycicek *et al*., 2006; Sarker *et al*., 2018).

Every one of these agents reduced the bacterial count to some extent, according to the results of how well vinegar and salt solutions reduced the bacterial load of the fruits and vegetables that were treated. At a 15% concentration, vinegar lowered the bacterial count to 0.77–1.49 log at zero (0) time. After 10 minutes of exposure, the concentration of vinegar at 25% showed the greatest reduction in bacterial count, dropping to 1.61–1.91 log. Although statistically insignificant (P>0.05), the bacterial loads found in this investigation were significantly reduced, as reported by Amoah *et al*. (2009) in their study on the microbial load decrease in vegetables washed in vinegar solution. Their research indicates that the effectiveness of vinegar in decreasing the bacterial population on fruits and vegetables was enhanced by increasing concentration and exposure time as observed in this study. According to Karapinar and Gonul (1992), inoculating parsley leaves with *Yersinia enterocolitica* reduced the number of CFU/g by 107 to 1 after 15 minutes of washing in a solution containing 40% vinegar or 2% acetic acid. Furthermore, after a 30-minute immersion in 5% acetic acid, no viable aerobic bacteria were found; whereas, a vinegar dip, which varied in terms of exposure duration and vinegar content, reduced the quantity of aerobic bacteria by 3 log10. Wright *et al*. (2000) found that, out of numerous treatments tested, a two-minute room-temperature dip in 5% acetic acid was the most successful in lowering populations of *E. coli* O157:H7 inoculated onto apple surfaces.

According to Parish *et al*. (2003), the type of treatment, the kind and physiology of the target microorganisms, the produce surface features, the length of exposure, the concentration of the sanitizer, pH, and temperature all affect how effective the procedure is for reducing the bacterial load. The findings in this study, which showed a proportionate decrease in bacterial loads as vinegar concentration rose, might be explained by the potential pH drop brought on by higher vinegar concentration. Furthermore, prolonged exposure to this unfavourable pH may be the cause of the increasing decline in bacterial loads that occurs with an increase in exposure duration (Eni *et al*., 2010).

The results also indicate a little reduction in bacteria in the salt solution. The fruits and vegetables treated with 15% salt solution at the beginning of the experiment showed the least amount of bacterial reduction, with the bacterial load reduced by 0.50-1.23 log. The greatest reduction was observed with 25% salt concentration after 10 minutes of exposure time, which reduced the bacterial count by 0.94-1.59 log. The findings of this study corroborated prior research showing vinegar to be more effective than salt water and tap water to be least effective, showing

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that washing or rinsing fruits and vegetables with vinegar solution was more successful than with salt solution (Atter *et al.,* 2014). The higher effective nature of vinegar solution over salt solution in reducing the bacterial loads of fruits and vegetables observed in this study and similar reports (Raj *et al*., 2017; Davati, 2023; Harrison *et al*., 2023; Yangılar *et al*., 2023) could be attributed to increasing the acidity of the washed products making them unfavourable for growth of most food spoilage causing bacteria. According to Shalaby and El-Raliman (1995), washing or rinsing vegetables with a salt solution was more successful than using plain water. When used for washing cucumbers and spinach, the amount of germs lowered by one to two logarithmic units. According to Ajayi *et al*. (2017), vinegar has an acidic and bactericidal character, whereas salt has an alkaline and bacteriostatic nature. Additionally, most bacteria prefer an alkaline pH over an acidic pH (Eni *et al*., 2010). Beuchat (1998) highlighted the potential of organic acids in lowering the amounts of microbes on fruits and vegetables in a World Health Organization report. Overall, this study's findings proved that vinegar, or acetic acid, works well as a fruit and vegetable disinfectant.

CONCLUSION

The study's findings showed how well vinegar, or acetic acid, works to lower the bacterial loads in fruits and vegetables. For those who prepare and handle ready-to-eat fruits and vegetables, vinegar treatment is an easy and affordable way to disinfect since it may lower the risk of foodborne illnesses linked to contaminated fruits and vegetables. Depending on the amount utilized and the length of exposure, salt was found to somewhat lower the bacterial loads of fruits and vegetables.

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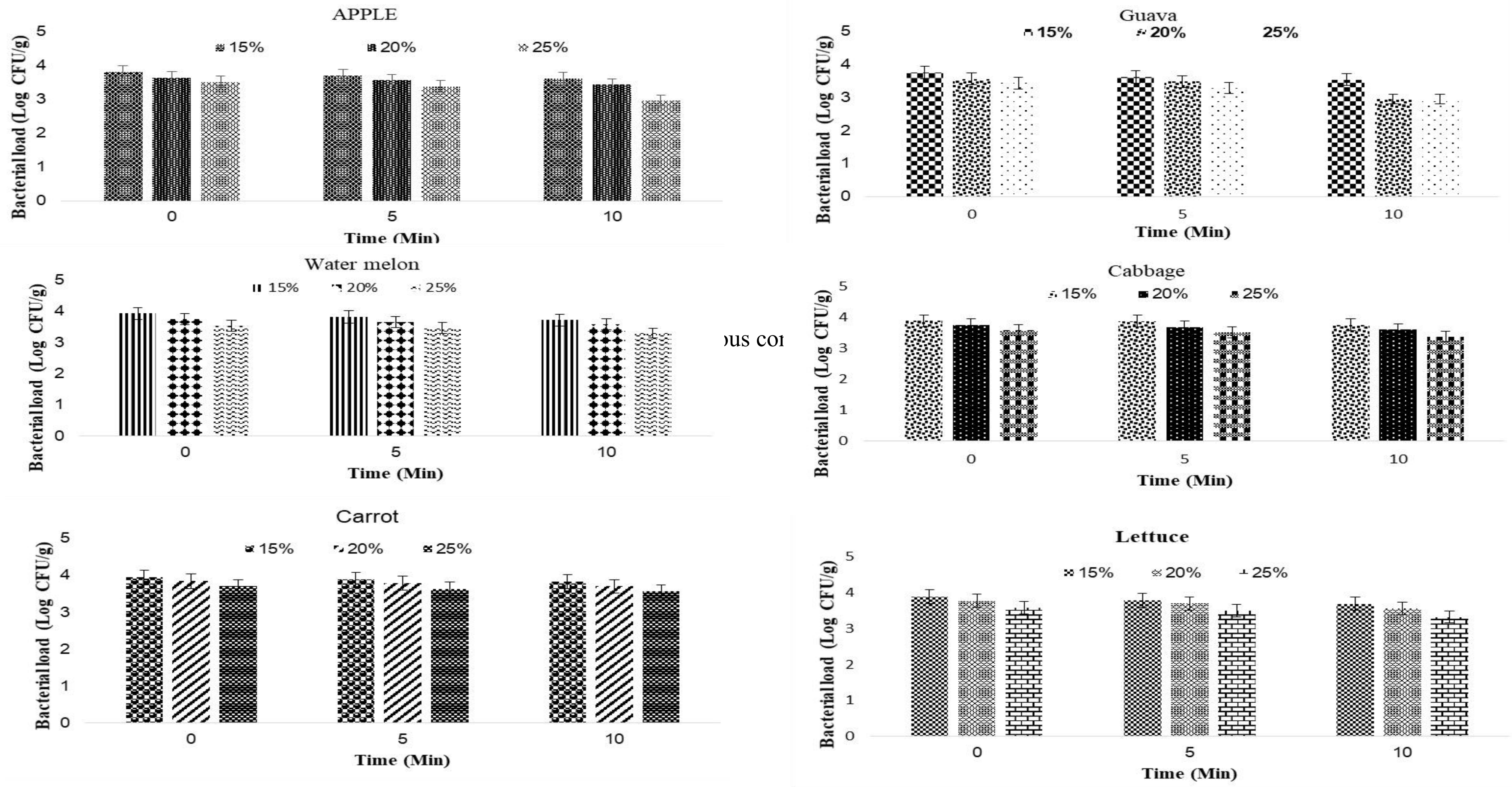

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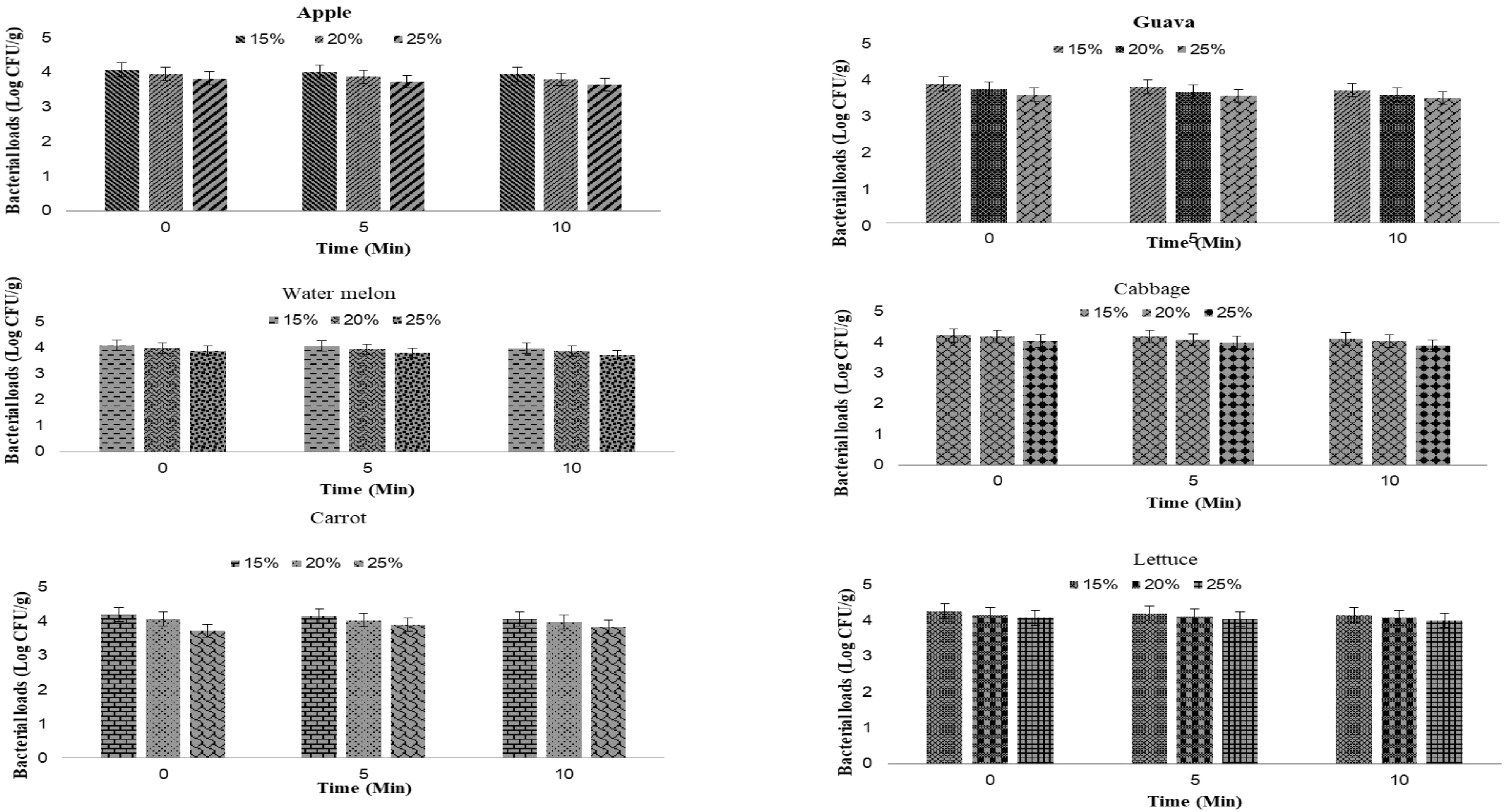

Figure 2: Bacterial loads of Fruits and Vegetables after treatment with various concentrations of salt in time intervals.

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