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Effectiveness of Chlorinated Water, Sodium Hypochlorite, Sodium Chloride and Sterile Distilled Water in Killing Pathogenic Bacteria on Fresh Produce

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Abstract · This study evaluated the efficacy of chlorinated water, sodium hypochlorite solution, sodium chloride solution and sterile distilled water in eliminating pathogenic bacteria on the surfaces of raw vegetables. Lettuce vegetables were dipped in different concentrations of chlorinated water, sodium hypochlorite solution, sodium chloride solution and sterile distilled water for 1,2,3,4 and 5 minutes and analyzed microbiologically. Treatments of the vegetables with chlorinated water, sodium hypochlorite solution, salt water and distilled water did not eliminate the pathogens, but reduced their populations. The bacterial loads were reduced by 38.18 – 69.83% (chlorinated water), 23.74 – 69.13% (sodium hypochlorite solution), 31.62 – 79.0% (salt water) and 14.95 – 42.59% (sterile distilled water). The coliform loads were reduced by 41.2 – 68.9% (chlorinated water), 22.2 – 55.6% (sodium hypochlorite solution), 36.0 – 90.0% (salt water) and 22.2% - 40.0% (sterile distilled water). This suggests that very high concentrations of salt water could be useful in reducing pathogenic microorganisms in fresh produce.

Keywords · Pathogenic bacteria · Bacterial loads · Vegetables

Efficacité de l'Eau Chlorinatée, Hypochlorite de Sodium, Chlorite de Sodium, et de l'Eau Désinfectée et Distillée dans la Stérilisation contre les Bactéries Pathogènes sur les Produits Agricoles Frais · Résumé · *Cette étude a évalué l'efficacité de l'eau chlorinateée, de l'hypochlorite de sodium, de la solution de chlorite de sodium, et de l'eau désinfectée et distillée dans l'élimination de bactéries pathogènes sur les surfaces de fraîches végétales. Les betteraves blanches ont été plongées dans différentes concentrations d'eau chlorinatée, solution d'hypochlorite de sodium, solution de chlorite de sodium, et d'eau désinfectée et distillée pour 1,2,3,4 et 5 minutes puis analysées microbiologiquement. Les traitements de végétales avec l'eau chlorinatée, la solution d'hypochlorite de sodium, l'eau salée et l'eau distillée n'ont guère éliminé les pathogènes, mais réduits leur populations. Les populations bactériennes ont été réduites par 38.18 – 69.83% (l'eau chlorinatée), 23.74 – 69.13% (la solution d'hypochlorite de sodium), 31.62 – 79.0% (l'eau salée) et 14.95 – 42.59% (l'eau désinfectée et distillée). La population de coliforme a été réduite par 41.2 – 68.9% (l'eau chlorinatée), 22.2 – 55.6% (la solution d'hypochlorite de sodium), 36.0 – 90.0% (l'eau salée) et 22.2% - 40.0% (l'eau désinfectée et distillée). Ceci suggère qu'une forte concentration d'eau salée pourrait être utile dans la réduction de microorganismes pathogènes sur les produits agricoles frais.* ***Mots Clé ·*** *Bactéries pathogènes, populations bactériennes, Végétales*

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Introduction

Recent outbreaks of food-borne diseases attributed to the consumption of fresh farm produce have increased awareness about the potential contamination caused by microbial pathogens along the food production chain. Several outbreaks of human gastroenteritis have been linked to the consumption of fresh vegetables contaminated with pathogenic organisms. Sources of pre-harvest contamination of farm produce include faeces, soil, irrigation water, improperly composted manure, air, wild and domestic animals and human handling (Beuchat, 1996a; Wachtel *et al.,* 2002).

To render farm produce pathogen-free, sanitizing procedures for eliminating pathogenic microorganisms from produce should be employed. Hurst and Schuler (1992) stated that it is management's responsibility to ensure that all employees handling fresh produce be thoroughly trained to understand the basic principles of food spoilage and food borne illness. However, efforts should be made to reduce the load of pathogenic microorganisms on farm produce at any points during growing, processing, storage and distribution. Thorough washing of raw vegetables and fruits with water can effectively remove soil and other debris from fresh fruits and vegetables, but should not be relied upon to completely remove microorganisms (Beuchat, 1996b). Although many methods of sanitation have been adopted, none has a broad-spectrum efficiency as chlorine. The use of chlorinated water will reduce microbial populations on raw vegetables but not totally eliminate them. Antimicrobial activity depends on the amount of freely available chlorine (hypochlorous acid) in water that comes in contact with microbial cells (Beuchat, 1996a).

Moreover, the effectiveness of Trisodium phosphate (TSP) in wash water in inactivation of *Salmonella montevideo* on the surface and in core tissue of unwashed tomatoes has been evaluated and the populations of microorganisms were significantly reduced. Ozone, at microgram per ml concentration, is also lethal to a wide variety of microorganisms and can be used as a sanitizer of fruits and vegetables. It may also be used to decontaminate wash water used in produce processing operations. Gamma irradiation is highly effective in killing microorganisms in foods including fresh fruits and vegetables (Monk *et al.,* 1995). The use of irradiation to preserve mushrooms, strawberries, grapes and other berries has been reported to be effective (Berrang *et al*., 1989). Although many sanitizers have been evaluated for their effectiveness in killing pathogenic microorganisms on different types of produce, fewer are available to the consumers in Nigeria. Although, washing of produce with tap water may remove soils and other debris, it cannot be relied upon to remove microorganisms completely and may result in cross-contamination of food preparation surfaces, utensils and other foods. Therefore, in order to minimize the health hazards associated with the consumption of fresh fruits and vegetables, there is need to develop effective antimicrobial treatments for the inactivation of pathogenic microorganisms on foods, so that the spread of the food-borne diseases via consumption of raw vegetables will be prevented as well as to protect the health of our communities. This study evaluated the effectiveness of some sanitizing agents namely, sodium hypochlorite solution, chlorinated water, salt water and sterile distilled water in eliminating the pathogenic microorganisms associated with raw vegetables.

Materials and Methods

Preparation of Washing Sanitizers

The washing sanitizers used were chlorinated water, sodium hypochlorite (NaOCl) solution, sodium chloride solution (salt water) and sterile distilled water. 100ppm, 200ppm and 300ppm of chlorinated water were prepared by dissolving 0.10g, 0.20g and 0.30g of chlorine powder in 100ml of distilled water respectively. 1%, 2% and 3% of sodium hypochlorite solution were prepared by mixing 1.0ml, 2.0ml and 3.0ml of sodium hypochlorite in 100ml of distilled water respectively. 2%, 4%, 6%, 8% and 10% sodium chloride solution were prepared by dissolving 2.0g, 4.0g, 6.0g, 8.0g and 10.0g of sodium chloride salt in 100ml of distilled water respectively.

Treatment of the Vegetables

Samples of lettuce vegetable (planted and irrigated with polluted stream water for 60days) were collected and washed with different concentrations of chlorinated water, sodium hypochlorite solution, sodium chloride solution and sterile distilled water. 1.0g of vegetable sample was separately dipped in 100ppm, 200ppm and 300ppm of chlorinated water for 1,2,3,4, and 5 minutes; in 1%, 2% and 3% of sodium hypochlorite solution for 1,2,3,4 and 5 minutes, in 2%, 4%, 6%, 8% and 10% sodium chloride solution for 1,2,3,4 and 5 minutes; and in sterile distilled water for 1,2,3,4 and 5 minutes. The treated samples were rinsed thoroughly with sterile distilled water and analyzed microbiologically. Control samples were not washed.

Microbiological Analysis

One gram of washed and control samples of the vegetable was separately chopped with 10.0ml of sterile peptone water. After then, series of dilutions were made by mixing 1.0ml of the suspension in 9.0ml of sterile peptone water to obtained 10^{-1} dilution. The dilution was then made to 10^{-2}, 10^{-3} and 10^{-4}. 1.0ml of each diluent was inoculated on the Plate count agar (PCA) and MacConkey agar. The pour plate method was adopted. The plates were incubated at 37^0C for 24-48hours. The effects of these agents were compared with control. Effect was studied on the basis of % decrease in microbial load.

Statistical Analysis

All plates were prepared in triplicates. Plate count and coliform count data were expressed as the log_{10} mean cfu/g values ± standard deviations obtained from triplicated plates. Significant differences in plate count and coliform count data were established by Duncan Multiple Range test at 5% level of significance.

Results

Data on the effectiveness of chlorinated water, sodium hypochlorite solutions, sodium chloride solutions and sterile distilled water in inactivating bacteria on the vegetables are shown in tables 1, 2, 3, 4, 5, 6 and 7. All the sanitizing agents showed reduction in bacterial and coliform loads. Washing the raw vegetable with 10% sodium chloride solution for 5 minutes achieved a greater percentage reduction (79.0%) of total bacterial load (table 5), followed by washing it with 300ppm chlorinated water for 5minutes (69.83%) while washing the raw vegetable with sterile distilled water achieved the lowest percentage reduction of total bacterial load (14.95%). Also, washing the vegetable with sterile distilled water and 1% sodium hypochlorite solution for 1 minute reduced the coliform bacteria by 22.2% while 90% reduction of coliform bacteria was achieved when washed with 10% sodium chloride solution for 5 minutes (table 5). These results demonstrated that 10% sodium chloride solution has anti-microbial activity that exceeded the anti-microbial activities of 100ppm, 200ppm and 300ppm chlorinated water, 1%, 2%, and 3% sodium hypochlorite solutions and sterile distilled water. 10% sodium chloride solution is therefore recommended for washing raw produce before consumption.

Table 1: Effect of Chlorinated Water on Bacterial load of vegetable sample

Concentration (ppm)	Total bacterial load after treatment (log_{10}cfu/g ± S.D)				
	Time (min)				
	1.0	2.0	3.0	4.0	5.0
100	5.89 ± 0.02 ^{a} (38.18)	5.83 ± 0.06^{ab} (47.67)	5.81 ±0.06^{ab} (49.62)	5.72 ± 0.04^{b} (59.23)	5.68 ± 0.05^{b} (62.50)
200	5.88 ± 0.04^{a} (40.19)	5.79 ±0.05^{ab} (51.40)	5.77 ± 0.0 ^{ab} (54.43)	5.67 ± 0.0^{b} (63.06)	5.65 ± 0.11^{b} (64.84)
300	5.81 ±0.01^{a} (49.01)	5.74 ±0.20^{ab} (55.92)	5.62 ± 0.1^{b} (67.69)	5.60 ± 0.13^{b} (69.16)	5.59 ± 0.04^{b} (69.83)
Control	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}

Note: Means with the different letters along the rows are significantly different ($P<0.05$)
Values in brackets are % reduction in bacterial loads.

Table 2: Effect of chlorinated water on coliform load of vegetable sample

Concentration (ppm)	Total coliform load after treatment (log_{10}cfu/g ± S.D.)				
	Time (min)				
	1.0	2.0	3.0	4.0	5.0
100	5.20 ± 0.08^{a} (41.2)	5.18 ± 0.00^{ab} (43.60)	5.15 ± 0.00^{ab} (48.0)	5.10 ± 0.06^{ab} (53.70)	5.06 ± 0.00^{b} (57.80)
200	5.17 ± 0.00^{a} (44.0)	5.15 ± 0.10^{a} (48.10)	5.09 ± 0.05^{ab} (54.5)	5.03 ± 0.03^{b} (60.20)	4.99 ± 0.05^{b} (63.60)
300	5.11 ± 0.07^{a} (51.9)	4.99 ± 0.05^{ab} (63.60)	4.98 ± 0.01^{b} (65.0)	4.92 ± 0.03^{b} (68.9)	4.92 ± 0.03^{b} (68.9)
Control	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}

Note: Means with the different letters along the rows are significantly different ($P<0.05$).
Values in brackets are % reduction coliform loads.

Table 3: Effect of Sodium hypochlorite (Naocl) solution on Bacterial load of vegetable Sample

Concentration (%)	Total bacterial load after treatment (log_{10}cfu/g ± S.D.)				
	Time (min)				
	1.0	2.0	3.0	4.0	5.0
1.0	5.99 ± 0.11^{a} (23.74)	5.91 ± 0.06^{ab} (36.35)	5.86 ± 0.02^{ab} (43.10)	5.81 ± 0.10^{b} (50.09)	5.74 ± 0.00^{b} (57.41)
2.0	5.95 ± 0.00^{a} (29.81)	5.89 ± 0.06^{ab} (39.63)	5.85 ± 0.03^{ab} (44.60)	5.72 ± 0.05^{bc} (58.78)	5.64 ± 0.02^{c} (65.71)
3.0	5.91 ± 0.30^{a} (36.60)	5.83 ± 0.01^{ac} (46.76)	5.70 ± 0.01^{bc} (60.48)	5.63 ± 0.10^{b} (66.90)	5.60 ± 0.03^{b} (69.13)
Control	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}

Note: Means with the different letters along the rows are significantly different ($P<0.05$).
Values in brackets are % reduction bacterial loads

Table 4: Effect of Sodium hypochlorite (NaOCl) solution on Coliform load of vegetable sample

Concentration (%)	Total coliform load after treatment (Log10 cfu/g ± S.D.)				
	Time (min)				
	1.0	2.0	3.0	4.0	5.0
1.0	5.32 ± 0.04^{a} (22.20)	5.28 ± 0.02^{ab} (30.0)	5.20 ± 0.10^{ab} (41.20)	5.17 ± 0.00^{b} (45.5)	5.15 ± 0.00^{b} (47.10)
2.0	5.30 ± 0.01^{a} (25.40)	5.26 ± 0.02^{ab} (33.3)	5.18 ± 0.20^{abc}(44.00)	5.15 ± 0.03^{bc}(48.10)	5.09 ± 0.03^{c}(54.20)
3.0	5.26 ± 0.00^{a} (33.30)	5.20 ± 0.02^{ab} (41.2)	5.13 ± 0.01^{ab} (50.00)	5.09 ± 0.01^{b} (54.50)	5.08 ± 0.01^{b} (55.60)
Control	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}	5.43 ± 0.10^{a}

Note: Means with the different letters along the rows are significantly different ($P<0.05$).
Values in brackets are % reduction coliform loads.

Table 5: Effect of Sodium Chloride Solution on Bacterial Load of Vegetable Sample

Concentration (%)	Total bacterial load after treatment (Log_{10}cfu/g ± S.D.)				
	Time (min)				
	1.0	2.0	3.0	4.0	5.0
2.0	5.94 ± 0.01^{a} (31.6)	5.85 ± 0.02^{ab} (44.90)	5.79 ± 0.02^{ab} (51.58)	5.78 ± 0.01^{ab} (53.45)	5.77 ± 0.00^{b} (54.50)
4.0	5.84 ± 0.10^{a} (45.13)	5.82 ± 0.00^{a} (48.50)	579 ± 0.04^{a} (51.64)	5.75 ± 0.06^{a} (55.91)	5.70 ± 0.03^{a} (61.25)
6.0	5.81 ± 0.01^{a} (49.20)	5.77 ± 0.00^{ab} (54.00)	5.71 ± 0.00^{ab} (60.40)	5.64 ± 0.02^{b} (66.20)	5.62 ± 0.10^{b} (67.60)
8.0	5.72 ± 0.05^{a} (59.00)	5.69 ± 0.05^{ac}(61.60)	5.67 ± 0.01^{abc} (63.80)	5.55 ± 0.02^{bc} (72.20)	5.51 ± 0.06^{b} (74.80)
10.0	5.67 ± 0.01^{a} (63.60)	5.58 ± 0.03^{ab} (70.60)	5.46 ± 0.03^{b} (77.50)	5.44 ± 0.02^{b} (78.90)	5.43 ± 0.10^{b} (79.0)
Control	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}	6.11 ± 0.03^{a}

Note: Means with the different letters along the rows are significantly different ($P<0.05$).
Values in brackets are % reduction bacterial loads.

Table 6: Effect of Sodium Chloride Solution on Coliform Load of Vegetable Sample

Concentration (ppm)	Total coliform load after treatment (Log_{10}cfu/g ± S.D)				
	Time (min)				
	1.0	2.0	3.0	4.0	5.0
2.0	5.24 ± 0.01[a] (36.0)	5.20 ± 0.06[ab] (40.70)	5.20 ± 0.01[ab] (40.70)	5.18 ± 0.01[ab] (44.40)	5.09 ± 0.01[b] (54.40)
4.0	5.19 ± 0.04[a] (42.10)	5.15 ± 0.01[ac] (48.0)	5.10 ± 0.02[abc] (53.40)	5.07 ± 0.00[abc] (56.00)	5.01 ± 0.05[b] (62.20)
6.0	5.11 ± 0.00[a] (52.00)	5.08 ± 0.00[ac] (55.60)	5.05 ± 0.00[ac] (58.60)	4.98 ±0.00[abc] (64.70)	4.91 ± 0.02[b] (70.0)
8.0	5.11 ± 0.01[a] (52.00)	5.07 ± 0.03[a] (56.0)	4.79 ± 0.03[b] (77.0)	4.78 ± 0.03[b] (77.8)	4.74 ± 0.04[b] (79.8)
10.0	5.07 ± 0.10[a] (56.0)	5.0 ± 0.00[a] (63.0)	4.78 ± 0.03[b] (77.8)	4.68 ± 0.04[b] (82.20)	4.43± 0.04[c] (90.0)
Control	5.43 ± 0.10[a]	5.43 ± 0.10[a]	5.43 ± 0.10[a]	5.43 ± 0.10[a]	5.43 ± 0.10[a]

Note: Means with the different letters along the rows are significantly different ($P<0.05$).
Values in brackets are % reduction coliform loads.

Table 7: Effect of Sterile Distilled Water on Bacterial and Colifrom loads of vegetable sample

	Time (min)					
	1.0	2.0	3.0	4.0	5.0	Control
Total bacterial load after treatment (log_{10}cfu/g ±S.D)	6.04 ± 0.05[ab] (14.95)	5.98 ± 0.03[ab] (25.20)	5.88 ± 0.10[b] (40.10)	5.87 ± 0.01[b] (42.59)	5.87 ± 0.01[b] (42.59)	6.11 ± 0.03[a]
Total coliform load after treatment (log_{10}cfu/g ±S.D)	5.32 ± 0.04[ab] (22.20)	5.28 ± 0.01[ab] (29.40)	5.24 ± 0.01[b] (35.11)	5.21 ± 0.02[b] (40.00)	5.21 ± 0.02[b] (40.00)	5.43 ± 0.10[a]

Note: Means with the different letters along the rows are significantly different ($P<0.05$).
Values in brackets are % reduction loads.

Discussion

Treatment of vegetable plants with 100ppm, 200ppm, and 300ppm chorine; 1%,2% and 3% sodium hypochlorite (NaOCl) solutions; 2%, 4%, 6%, 8% and 10% sodium chloride solutions and sterile distilled water failed to completely eliminate bacteria from the vegetables, but reduced their populations. The antimicrobial agents showed 14 – 90% reduction in a bacterial load. Effect of 10% sodium chloride solution showed maximum decrease of microbial load (31-90%) reduction, 300ppm chlorinated water being the second effective compound for decreasing microbial load (38 – 69.83%) and sterile distilled water showed minimum decrease of microbial load (14 - 42.59%). The difference which was statistically significant at $p < 0.05$ is an indication that higher concentration of salt water will reduce the bacterial and coliform loads further. These observations corroborate previous findings that demonstrated the limited effectiveness of chlorine and distilled water in the decontamination of farm produce (Beuchat and Ryu, 1997; Li *et al.*, 2001; Bari *et al.,* 2002; Solomon *et al.,* 2002 and Joshi and Patel, 2005). Although other sanitizing agents such as chlorine dioxide and ozonated water are available, chlorine at a concentration of 200ppm is currently the chemical sanitizer that is mostly used in the produce industry. Though chlorine is highly effective against *E. coli* in aqueous systems, its efficacy is greatly reduced on raw fruits and vegetables (Rice *et al.,* 1999). Beuchat (1998) attributed the loss of activity to the interaction between chlorine and the plant organic materials such as plant tissues.

In Nigeria, because of high cost of chlorine and sodium hypochlorite, people usually wash their vegetables and fruits with either ordinary water or low concentration of salt water. This study showed that such vegetables and fruits will still be carrying a lot of microorganisms that are capable of causing food poisoning and intestinal diseases. It is then suggested that vegetables to be consumed raw should be washed thoroughly with very high concentration of salt water, if chlorine and sodium hypochlorite are not available, instead of ordinary water.

Conclusion

This study revealed that pre-treatment of fresh produce by various anti-microbial agents should be done in order to decrease the density of microbial contaminant from the surface of the fresh produce and to minimize the chances of food-borne out breaks in our society. It is therefore suggested that vegetables and fruits to be consumed raw should be washed with high concentration of sodium chloride solution or chlorinated water before being consumed, so as to remove most pathogens present on the vegetables and fruits.

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