Effects of Vinegar (5% Acetic Acid) Treatments On Reduction of Microbial Load On Lettuce

Mohammed Murtala Kyari
Department of Animal Health and Production Technology,
Federal Polytechnic Bali, Taraba State
mmkyari@gmail.com 07030610999

Abstract

Vegetable promotes good health but harbor a wide range of microbial contamination. This study was carried out to compare the effectiveness of two commercially available vinegars labeled as (X and Y) at different concentration in decontamination of lettuce sold in Ahmadu Bello University, Zaria. Thirty (30) samples of lettuce vegetable were purchased from two different sell points in the University. The samples were analyzed using standard bacteriological method to enumerate total aerobic plate count before and after washing with vinegars at different concentrations. In addition Minimum Inhibition Concentration (MIC) was used to measure zone of bacterial inhibition. The result showed that the mean total aerobic plate count of 5.64 log_{10} cfu/g before washing with vinegar. After treatment with vinegar X the mean counts were 3.79, 4.95, and 5.28 log_{10} cfu/g for different concentration 1:1, 1:2 and 1:3 respectively. While after treatment with vinegar Y the mean total aerobic plate counts were 4.09, 5.27, and 5.39 log_{10} cfu/g at different concentration 1:1, 1:2, and 1:3 respectively. Two genera of bacteria were isolated with the percentage isolation rate of 22 (75%) for proteus and Eschericia coli 8 (25%). From the MIC mean zone of inhibition of 16.6mm and 13.4mm for the stock solution X and Y respectively were recorded showing a slight difference compared to one obtained after treating the lettuce with higher concentration above the manufacturers recommended concentration. The use of vinegar in washing fruits and vegetable using the appropriate concentration is recommended as shown by the result.

Key words: Vinegar, Minimum inhibition concentration, Lettuce, Vegetables.

Introduction

The importance of fruits and vegetables to human cannot be over emphasized as they are high in fiber, vitamins, water, and minerals, varying proportions of sugar, proteins and various phytochemicals such as flavonoid, saponin, tannin and anthocyanin. Example of those fruits and vegetables are apple, banana, pineapple, paw-paw, oranges, lettuce, spinach, cabbage, cauliflower (Gruda, 2005).

Vegetable salad is a very common food accompaniment in Nigeria. The vegetables that usually make up this recipe include tomatoes, cucumber, carrots, green chili, cabbage and lettuce. They are sold in almost every market, and can be seen hawked around by traders (Oji, p.c., 2016). Fruits and vegetables have been identified as significant sources of pathogens and chemical contaminants (Uzeh *et al*., 2009). As a result, environmental and food microbiologists have continued to identify and suggest control measures for hazards at all stages in the supply chain (Johngen, 2005). Micro-organisms capable of causing human illness such as *Salmonella spp, Eschericia coli, proteus spp, Aeromonas hydrophila, Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella spp*. have also been isolated in lettuce and salad vegetables (Francis *et al*., 1999).

Khan *et al*. (1992) reported that bacterial contamination results from various unsanitary cultivation and marketing practices. In another study, Tambekar *et al*. (2006) reported that bacterial contamination of salad vegetables was linked to the fact that they are usually consumed without any treatment. These vegetables can become contaminated with pathogenic microorganisms during harvesting, through human handling, harvesting equipment, and transport containers. Pathogens from the human and animal reservoir as well as other environmental pathogens can be found at the time of consumption. Although spoilage bacteria, yeasts and mould dominate the micro flora on raw fruits and vegetable, the occasional presence of pathogenic bacteria, parasites and viruses capable of causing human infections has also been documented (Hassan *et al*., 2006).

Coliforms are facultative anaerobic Gram negative rods belonging to the family *Enterobacteriacaea*. They are known contaminants of food and water, causing various intestinal and extra-intestinal infections such as urinary, central nervous system and respiratory tract infections (John, 2007). The presence of *E. coli*, *Enterobacter sp*, *Salmonella sp*, *Shigella sp* and *Pseudomonas aeruginosa*, has been reported in salad vegetables (Khan *et al*., 1992; Tambekar, 2006). Mehmet and Aydin (2008) also reported the presence of *E. coli* in some green leafy vegetables.

Due to the favourable climatic condition for cultivation of salad vegetables, as well as the cultural practice of dwellers in Northern Nigeria, the consumption rate of these vegetables is higher than in other regions in Nigeria.

Vinegar (Acetic acid) is well recognized as a cleaning agent. It is especially effective in removing inorganic soils and mineral deposits such as hard water films. It is also effective against a broad range of bacteria, destroying or reducing these organisms to acceptable levels. Acetic acid in small amounts and at relatively high pH values proved more toxic to representative bacteria, yeast and mold than lactic or hydrochloric acid. Not only can acetic acid inhibit and destroy microorganisms when used in sufficiently high concentrations, it also aids materially in reducing thermal death rates of bacteria when present in sub-lethal concentrations (Levine and Fellers,1990).Most manufacturers recommend one part of vinegar to be diluted in two part of water. There is

paucity of information obtained on the effectiveness of commercially available vinegar in the market.

Objectives

- To assess the microbial load on lettuce sold in Ahmadu Bello University, Zaria, Nigeria.
- To compare the effectiveness of two different available vinegar solutions at different concentrations on the microbial load of lettuce.
- To suggest the appropriate concentration that is more effective in reducing the microbial load.

Materials and Methods

Sample collection

Five (5) Samples per week at different sales point for a period of Six (6) weeks a total of 30 samples of fresh lettuce were collected from Ahmadu Bello University Zaria community market and ICSA. The lettuce samples were collected in a sterile polythene bags and transported to the laboratory immediately for analysis.

Laboratory procedures

Unwashed (Untreated) Samples

10 grams of lettuce samples were weighed (before washing) and homogenized with 90mls of 0.1% bacteriological peptone using stomacher. Subsequently,0.1ml of each homogenate were serially (100 fold) diluted and 0.1ml of 10^{-4} dilution were pipette into solid medium(Nutrient agar) following surface plating techniques and inverted, incubated at 37^{oc} for 24-48 hour for bacterial counts. The colonies that appeared were preserved by sub culturing on nutrient agar slant for further biochemical test.

Vinegar (5% Acetic acid) Treatment of Lettuce

To determine the effect of various concentrations of acetic acid solution on the microbial load of lettuce, with the same samples (i.e unwashed). 10grams of each sample was weighed and washed vigorously in different concentration of 5% acetic acid (I .e 1:1, 1:2 and 1:3) ratio of acetic acid to water, with the manufacturer recommended concentration of 1:2. Then the washed samples were homogenized with 90mls of 0.1% bacteriological peptone, 0.1ml of each homogenate was serially(100 fold) diluted then 0.1ml of 10^{-4} dilution were pipette into nutrient agar following surface plate technique and inverted then inoculated at 37^{0c} for 24 hours for bacterial counts. Biochemical test namely: TSI, urea, citrate, Indole, Methyl red and volkes prosker, then motility were carried out for identification.

Minimum Inhibition Concentration was determined by Gel Diffusion Test:

The isolates were sub-cultured on nutrient agar, the colonies were picked with Pasteur loop and put into a 5ml of normal saline diluents in a screw cap test tube and eluted into the saline diluents by swirling the Pasteur loop into it. The sterile swab was removed and dipped into the diluents and excess saline removed by pressing against the inner side of the tube. The swab was smeared on the already prepared nutrient agar. Eight circular well were made on the agar with two of the wells located centrally, the wells were labeled as follows; two central wells contained the stock solutions X and Y while the other six contained different concentrations of vinegar X and Y (i.e 1:1, 1:2, 1:3), then incubated at 37^{oc} for 24hrs. The minimum zone of inhibitions for the different concentrations and stock solutions were measured in millimeters and recorded.

Data Analysis

Descriptive statistics was used to express the mean log and standard deviation of total aerobic plate count before and after washing with vinegar. Paired t-test was also employed to check the difference in mean before and after washing with vinegar X and Y.

RESULT

Table I: **Range count of microbial load on lettuce in log_{10} cfu/g for vinegar X**

CONCENTRATION	RANGE COUNT
PRE-TREATMENT	5.30 — 6.89
1:1	3.00 — 5.07
1:2	3.48 — 5.79
1:3	3.95 — 5.79

Table II: **Range count of microbial load on lettuce in log_{10} cfu/g for vinegar Y**

CONCENTRATION	RANGE COUNT
PRE-TREATMENT	5.30 — 6.89
1:1	3.0 — 5.19
1:2	3.69 — 5.70
1:3	3.78 — 5.87

Table III: Mean Zone Of Inhibition (Mean± Standard Deviation) Of Micro-Organism to Different Concentration of Vinegar X and Y

Vinegar Type	1:1	1:2	1:3	STOCK SOLUTION
X	16.6±0.87	13.33±1.09	9.73±1.02	17.86±0.82
Y	13.4±0.89	10.66±0.58	6.2±0.93	15.46±0.68

Table IV: Showing percentage of isolates biochemically characterized from the lettuce

ISOLATE	SAMPLE NUMBER (%)
Proteus spp	22(75)
E coli	8(25)

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Table V: Mean log_{10} cfu/g total aerobic plate count before and after treatment with commercial vinegar X (subjected to t-test paired two samples for means)

Concentration	Before Treatment	After Treatment
1:1	5.64±0.04	3.79±0.19
1:2	5.64±0.04	4.95±0.10
1:3	5.64±0.04	5.28±0.05

The decrease in the mean difference values after and before treatment was statistically significant ($P < 0.05$)

Table VI: Mean log_{10} cfu/g total aerobic plate count before and after treatment with commercial vinegar Y (subjected to t-test paired two samples for means)

Concentration	Before Treatment	After Treatment
1:1	5.64±0.04	4.09±0.20
1:2	5.64±0.04	5.27±0.06
1:3	5.64±0.04	5.39±0.07

The decrease in the means difference values after and before treatment was statistically significant ($P < 0.05$)

Discussion

Cenci-Goga *et al*. (2005) pointed out that total aerobic bacteria count was a good indicator of food safety. In this study the bacterial count obtained from lettuce sample before treatment with vinegar the minimum value of 5.64 log_{10} cfu/g(Table I and II) has exceeded the recommended World Health Organization (WHO, 1996) and International Commission on Microbiological Specifications for Food (ICMSF, 1998) standards of 10^3 CFU/g (for example, Log_{10} 3.0 CFU/g). This finding is as a result of common agricultural practice in the study area where contaminated water with micro-organism is used in the irrigation of farm fields to produce vegetables. Also indicated when non-properly composite manure are used in fertilizing the farm field contribute to microbial contamination of vegetables produced as indicated by other studies (Taban and Halkman, 2011; Adjrah et al 2013). However Yeboah-manu has reported the total aerobic count which is higher than the present study with values ranged from 8.54—8.69 log_{10} cfu/g for lettuce sold around the university of Ghana, he gives similar reasons of used of contaminated irrigated water with micro-organism in the farm fields.

When result of total aerobic plate count before and after treatment with two different commercial vinegar was subjected to simple t-test there were statistically significant different in the microbial load after treatment with vinegar. However this indicates that the vinegar is effective against the bacteria.

As shown in this study, increase in concentration of vinegar had marked effect on bacterial load as increase in dilution reduced the effect on the bacterial organism. Vinegar X has more effect on the bacteria as compare to vinegar Y.

The detection of *E coli* in this study (Table IV) showed poor hygienic standard in the handling of this salad vegetables or it could be from contamination during harvest. Presence of *E coli* indicate recent contamination by faecal matter and possible presence of other enteric pathogens known to be causative agent of food borne gastroenteritis and bacterial diarrhea diseases (Adebayo-Tayo *et al*. 2012). Also reported by Lawan *et al* (2015) in Zaria STEC was isolated from cabbage sampled from the farm irrigated from stream contaminated with Abattoir effluent. The lettuce that was used in this study originated from the same farm where STEC was isolated in cabbage.

The Minimum inhibition concentration (MIC) technique indicated that the vinegar appeared to inhibit bacterial growth at 24 hours. Also increasing concentration had marked effect on the inhibition, this was evident for mean zone of inhibition obtained with the highest concentration (i.e 1:1) (Table III) for vinegar X and Y respectively having a slight different with the mean zone of inhibition of the stock solution (17.86mm and 15.46mm for X and Y respectively). While on the other hand the manufacturers recommended concentration of 1:2 having 13.33mm and 10.66mm as zone of inhibition with a wide difference compare to the values obtained with stock solution. This has shown that the higher concentration of 1:1 have greater effect on the microbial load almost similar to the stock solutions, the manufacturers recommended concentration (1:2) is not as effective compare to the one used in this study (Table I and II), meanwhile the acetic is not known to have any effect on the body at the concentration that was used which is higher than the manufacturers recommended concentration.

Conclusion

In conclusion use of high concentration of 1:1 in the study above the manufacturer's recommendation of 1:2 indicated more effective in reduction of microbial load on lettuce.

Recommendation

- The use of vinegar in washing fruits and vegetable using the appropriate concentration is recommended as shown by the result.
- Recommend that more vinegar in the market should be evaluated.
- Recommend that manufacturers should review their dilution

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