The Effectiveness of Water, Salt and Vinegar in Reducing the Bacteria Population in Fresh Green Cabbage.

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Abstract

Green cabbage has great nutritional and therapeutical values and is usually used as the main ingredient for preparing fresh vegetable salad for consumption. The aim of this work was to identify the bacteria population in different sections of green cabbage heads obtained from some selected markets in Accra and also to determine the most appropriate method for washing to reduce the bacteria population prior to consumption. In the investigation, samples were obtained from five open markets in the Greater Accra Region of Ghana. Fresh leaves taken from the outer, middle and inner sections of the samples were analyzed for aerobic mesophilic organisms, coliforms and Escherichia coli. They were then washed with tap water, 5% salt solution or 50% vinegar solution for five minutes, and the effectiveness of the treatments in reducing the bacteria population determined. Results from the analysis showed that the outer sections of fresh green cabbage heads had high microbial load followed by the middle sections whilst the inner sections were almost sterile. The aerobic mesophilic counts ranged from 10^6–10^8 CFU/g for the untreated outer section of the samples, 10^4–10^6 CFU/g for the untreated middle sections, and 10^1–10^2 CFU/g for the untreated inner sections. Coliforms ranged from 10^4–10^6 CFU/g for the untreated outer section of the samples, 10^2–10^4 CFU/g for the untreated middle sections and 10^1 CFU/g for the untreated inner sections. E.coli ranged from 10^2–10^3 CFU/g for the untreated outer section of the samples and 10^1 CFU/g for the untreated middle sections. E. coli was however not detected in the untreated inner sections of the cabbage heads analyzed. The pathogens Bacillus cereus and Staphylococcus aureus were also identified on the samples from the five markets. The treatment with 50% vinegar solution was most effective in reducing the bacteria population on the samples analyzed followed by 5% salt solution and tap water the least effective. The outcome of this work serves as a basis for creating awareness against the use of ordinary tap water by caterers and promote the use of vinegar or salt solutions as a means of reducing bacteria population in freshly served cabbages.

Keywords: Cabbage, Aerobic mesophiles, Coliforms, E. coli, Staphylococcus aureus, Bacillus cereus

1. INTRODUCTION

Vegetables, especially cabbages are widely cultivated and consumed in Ghana by people from all social backgrounds. However, cabbages are exposed to unhygienic practices throughout its production and supply chain. Cabbages may become contaminated at all stages during production and processing with possible sources being from the soil, faeces, water, animals, handling of the products, harvesting transportation and even by the sellers. In Ghana, harvested cabbage heads are usually transported by vehicles in sacks, baskets, basins or sometimes on the bare floor of these vehicles thereby exposing them to microbial contamination. Inappropriate use of manure as fertilizer also results in pathogens being transferred into vegetables. Ingham et al., (2004), revealed that vegetables grown in non-composted manure fertilized soil when analyzed recorded different levels of E. coli. Data on streams drains and other sources of polluted water used for vegetable production in the cities of Accra and Kumasi reported high levels of pollution with faecal coliforms. The use of such polluted irrigation water resulted in vegetable contamination with faecal coliforms (Mensah et al., 2000). Faecal coliforms concentrations in untreated municipal wastewater were given as 10^5–10^7 per 100ml (Gleick, 2000). Amoah et al., (2005), also conducted a study to determine and compare the level of exposure of the Ghanaian urban population to hazardous pesticide and faecal coliform contamination through the consumption of fresh vegetables produced in urban and peri urban smallholder areas with wastewater irrigation. The result showed

that the samples were faecally contaminated and carried faecal coliform populations with geometric mean values ranging from 4.0×10^3 to $9.3 \times 10^8 \text{ g}^{-1}$ wet weight and this exceeded recommended standards. Another study on the bacteria quality of vegetables in Kano also showed a high count of bacteria and coliform index (Aliyu et al, 2005). According to Harris et al., (2003), the pathogens in the intestinal tracts of animals and humans contaminate vegetables through faeces, sewage, untreated irrigation water or surface water. Contamination risks are therefore greatest for vegetables that are potentially eaten raw, especially when the vegetable grows close to or in the soil e.g. cabbage, lettuce, spinach, radish and carrots. Vegetables contaminated with enteric pathogens have been shown to cause severe to life threatening gastro-intestinal infections in consumers. For this reason, there have been a number of outbreaks of diseases linked to the consumption of such contaminated fresh vegetables. In view of this, there is therefore the need for interventions to prevent the spread of gastro-intestinal infections. The aim of this work was to identify the bacteria population in different sections of fresh green cabbage heads obtained from some selected markets in Accra and also to determine the most appropriate method for washing to reduce the bacteria population prior to consumption.

2. MATERIALS AND METHODS

2.1 Sampling and sample preparation

The study was undertaken in five open markets in the Greater Accra Region of Ghana. Thirty samples were obtained, placed into sterile bags and transported to the laboratory for analysis. In the laboratory, fresh leaves from the outer, middle and inner sections of the cabbages were aseptically removed and analyzed before and after washing in the various treatments for five minutes.

2.2 Homogenization and serial dilution

For the study 10 g of the cabbage samples was added to 90.0 ml sterile diluent containing 0.1% peptone, 0.8% NaCl, with pH adjusted to 7.2 and homogenized in a stomacher (Lad Blender, Model 4001, Seward Medical). After mixing for 30s at normal speed, the homogenate was serially diluted and directly inoculated into petri plates.

2.3 Microbiological Analysis

Aerobic mesophiles were enumerated by pour plate on Plate Count Agar (Oxoid CM325; Oxoid Ltd., Basingstoke, Hampshire, UK), incubated at 30 °C for 72 h in accordance with NMKL No. 86, 2006. Total coliforms and E. coli were enumerated by pour plate on Trypton Soy Agar (Oxoid CM131), pH 7.3 overlaid with Violet Red Bile Agar (Oxoid CM107), pH 7.4 and incubated at 37 °C for 24 h for total coliforms and at 44 °C for 24 h for E. coli. Colonies for total coliforms were confirmed with Brilliant Green Bile Broth (Oxoid CM31), pH 7.4 incubated at 37 °C for 24 h according to NMKL No. 44 (2004) and E. coli using EC Broth (Oxoid CM853), pH 6.9, followed by Trypton Water (Oxoid CM87), pH 7.5, all incubated at 44 °C for 24 h as described by (NMKL. No. 125, 2005). Staphylococcus aureus was determined by the spread plate method using Baird-Parker Agar (BP, CM 275 Oxoid Ltd, Hampshire, England.) with Egg Yolk Tellurite Emulsion (SR54) added and Blood Agar Base (BAB, CM 55 Oxoid Ltd, Hampshire, England.). Both media were incubated at 37°C for 48 h. Staphylococcus aureus counts were confirmed by biochemical tests according to NMKL Method No. 66, (2009). Bacillus cereus was also determined by the spread plate method as described by NMKL No. 67, (2010).

3.0 RESULTS AND DISCUSSION

The results of the bacteria loads from the various markets are presented in figure 1 to 3 whilst other isolated pathogens are presented in table 1 respectively. The results showed that generally the outer fresh green cabbage leaves carried the highest bacteria population followed by the middle section and the leaves from the inner section carried the least or no bacteria population. The mean bacteria population therefore decreased as it moved from the outer to the inner sections of the cabbage heads. The aerobic mesophilic counts ranged from $10^6 – 10^8$ CFU/g for the untreated outer section of the samples from the various markets, $10^4 – 10^6$ CFU/g for coliforms and $10^2 – 10^3$ CFU/g for E. coli. From the Madina market for example, the outer fresh green cabbage leaves carried mean aerobic mesophilic population of 7.3 x 10^8 CFU/g for untreated cabbage leaves, 3.1 x 10^8 CFU/g for tap water treated leaves, 8.9 x 10^7 CFU/g for salt solution (5%) treated samples and 4.6 x 10^5 CFU/g for vinegar solution (50%) treated leaves. For the samples obtained from the middle sections of the cabbage heads, the population of aerobic mesophiles was 6.9 x 10^6 CFU/g for untreated samples, 8.1 x 10^5 CFU/g for tap water, 5.5 x 10^4 CFU/g for salt solution treated and 4.2 x 10^3 CFU/g for vinegar solution treated samples. The samples obtained from the inner sections of the cabbage heads had low population of aerobic mesophiles of 9 x 10^1

CFU/g for untreated samples. No aerobic mesophilic population was isolated from the samples when treated with tap water, salt solution (5%) and vinegar solution (50%). Similar trends were recorded for the coliform and E. coli population found in the outer, middle and inner sections of cabbage samples obtained from Kaneshie, Makola, Adabraka and Mallam-Atta markets when untreated and treated with tap water, 5% salt solution and 50% vinegar solution respectively. From the study it was observed that the treatment with 50% vinegar solution was most effective in reducing the bacteria population on the samples analyzed followed by 5% salt solution and tap water, the least effective. Using the coliform population from the Kaneshie market for example can be used to support this observation. The untreated outer sections of the cabbages had a mean coliform population of 5.788 $\log_{10}$CFU/g whiles the untreated middle sections had a mean coliform population of 3.606 $\log_{10}$CFU/g at a p-value of 0.022 and 0.003 respectively at the 95% confidence interval. The mean coliform population however reduced drastically to 3.192 $\log_{10}$CFU/g for the untreated outer section whiles the untreated middle section also reduced to 1.433 $\log_{10}$CFU/g after treating with 50% vinegar. After treating the outer and middle sections of the cabbages with 5% salt solution, the coliform population reduced from 3.684 $\log_{10}$CFU/g for the outer sections to 2.185 $\log_{10}$CFU/g for the middle sections. Thus making 5% salt solution the second best in this analysis. The inner section of the cabbages did not contain any coliforms except for the samples obtained from the Madina market which had a negligible count but disappeared after washing with either 5% salt solution or 50% vinegar solution. Again, using the results of the aerobic mesophilic population obtained from the Mallam-Atta market for example showed that the untreated outer sections of the cabbages after washing with tap water still had about 86.5% of the bacteria population remaining on the cabbages. However, washing with 5% salt solution retained 4.38% of the bacteria population whilst 50% vinegar solution retained just 0.54%. Similar trends were observed from the other markets. The high bacteria population on the outer sections of the cabbages may be attributed to a number of factors. Since there is the need for the fertilization of the soil, some of these farmers apply poultry litter without adequate composting and this constitutes a potential source of food contamination especially leafy vegetables (Drechsel et al., 2000). Some of these cabbages fall on the ground during loading and offloading from the transporting vehicles. Lack of proper storage facilities in these markets also further contaminates the outer sections of the cabbages. Again, it is the outer section of the cabbages that the farmers, sellers and buyers usually handle coupled with the dusty environment they are exposed to may also contribute to the microbial load recorded. Comparatively, the middle section is less exposed to most of the bacteria contaminants. The inner section of the green cabbage had little or no bacteria population due to the nature of the cabbage shoot which is compressed, having the youngest and very pale greenish looking leaves at the centre of the cabbage head. Therefore the inner leaves are rarely exposed directly to the outside atmosphere, manure, handling from people etc. The isolation of Bacillus cereus from the samples from all the markets was expected considering the fact that Bacillus cereus has a wide distribution in nature, frequently isolated from soil and growing plants. It is also well adapted for growth in the intestinal tract of insects and mammals (Stenfors Arnesen et al., 2008). The poor handling and hygienic practices by both the farmers and sellers of cabbage heads makes it prone to Staphylococcus aureus as observed from some of the market centres. Apart from handlers of food, environmental surfaces and equipments can also be sources of contamination with Staphylococcus aureus (Sagoo et al., 2003). The presence of Staphylococcus aureus in foods can produce disease causing toxins which are heat stable and lead to food poisoning (Koneman et al., 1988). The bacterial pathogens found in this work agrees with Tambekar and Mundhada, (2006) who confirmed that food-borne bacterial pathogens commonly detected in fresh vegetables are coliform bacteria, E. coli, Staphylococcus aureus and Salmonella sp. Others also have reported the presence of microbial contamination in vegetables (Uzeh, et al., 2009; Halablab et al., 2011; Chaturvedi et al., 2013; Odu and Okomuda, 2013).

In view of the above, it is prudent that, the first few outer sections or layers of a fresh green cabbage head be removed and discarded as treatment with 50% vinegar solution will still give substantial bacteria counts. Even though the 50% vinegar solution was more effective in reducing the bacteria population, the 5% salt solution can be used in place because it is cheaper and more readily available. Vegetable growers should be educated on the hazards associated with using polluted or sewage irrigation water for irrigating vegetables in general on the consumer. If possible vegetable farmer's access to clean and safe water should be given more attention by the appropriate authorities. In addition, they should be educated on the safe use or application of manure, be it animal or poultry. Sellers must also be educated on good handling practices coupled with the use of safe water for washing and sprinkling to reduce the microbial load. Cabbage should be consumed by all in order to derive all desired benefits but not to acquire diseases such as diarrhea, urinary tract infections, nerve damage etc. as a result of the presence of pathogenic bacteria. For that matter, there is the need for public education or awareness creation on washing cabbage thoroughly with 50% vinegar or 5% salt solution. People should also know that washing with only tap water does not have any significant effect on the bacteria population as this is the practice

in most homes, canteens, food joints, restaurants etc.

4.0 CONCLUSION

The outer sections of fresh green cabbage heads available in the five selected markets in Accra have high bacteria counts as well as the middle sections whilst the inner sections are almost sterile. Using 50% vinegar solution to wash cabbages substantially reduces the bacteria population as well as 5% salt solution with the 50% vinegar solution being most effective. Ordinary tap water is not effective in reducing the bacteria population.

References

Aliyu, Y.U, Bassey, S.E and Kawo, A.H (2005). Bacteriological quality of vegetables sold in some shops around Kano metropolis, Nigeria. .Biological and Environmental Sciences Journal for the Tropics 2(1): 145 – 148.

Amoah, P. Brechsel, P. Abaidoo, R.C. and Ntow, W.J. (2005). "Pesticide and Pathogen

Contamination of Vegetables in Ghana's Urban Markets". Vol. 50. No.1. Springer-Verlag, New York.

Anonymous, 2006

Chaturvedi, M., Kumar, V., Singh, D. and Kumar, S. (2013). Assessment of microbial load of some common vegetables among two different socioeconomic groups. International Food Research Journal 20(5): 2927-2931.

Drechsel, P., Abaidoo, R.C., Amoah, P. and O.O. Cofie, (2000). Increasing use of poultry

manure in and around Kumasi, Ghana: Is farmers' race consumers' fate? Urban

Agricultural Magazine 2:25-27

Gleick, P.H., (2000). "The World's Water 2000-2001. The Biennial Report on Freshwater Resources".   Island Press, Washington. Pg. 152.

Halablab, M. A., Sheet, I.H. and Holail, H.M. (2011). Microbiological Quality of Raw Vegetables Grown in Bekaa Valley, Lebanon. American Journal of Food Technology 6: 129-139

Harris, L.J., Faber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.B., Garret, E.H. and Busta, F.F. (2003). Outbreaks associated with fresh produce: incidence, growth and survival of pathogens in fresh and fresh cut produce. Comprehensive Review of Food Science and Food Safety 2: 115-119.

Hatfield, Gabrielle (2004). Encyclopedia of Folk Medicine: Old World and New World Traditions. ABC-CLIO. pp. 59–60. ISBN 978-1- 57607-874-7.

Ingham, S.C., Losinski, J.A., Matthew P. Andrews, M.P., Breuer, J.E.,Jeffry R. Breuer, J.R., Wood, T.M., and Wright, T.H. (2004). Escherichia coli Contamination of Vegetables Grown in Soils Fertilized with Noncomposted Bovine Manure: Garden-Scale Studies. Appl Environ Microbiol. 70(11): 6420–6427.

Koneman, E., Stephen, D., Allen, V., Dowell, J., William, W., Sommers, J., and Washington, C. (1988). Diagnostic microbiology (3rd ed.), J. B. Lip-pincott, Washington. 312-316

Mathewson, J.J. (1998). Rapid healing of pepticulcers in patients receiving fresh cabbage juice.Calif. Med. 7:10-15.

Mensah, E., Amoah, P., Abaidoo, R.C. and P. Drechsel. (2001). Environmental concerns of (peri-) urban vegetable production – Case studies from Kumasi and Accra. In: Drechsel,P. and D. Kunze (eds.) Waste Composting for Urban and Peri-urban Agriculture -Closing the rural-urban nutrient cycle in sub-Saharan Africa. IWMI/FAO/CABI:Wallingford, p. 55-68.

Mensah, P., Yeboah-Manu, D., Owusu-Darko, K. and Ablordey A. (2002). Street foods in

Accra, Ghana: how safe are they? Bulletin of WHO 80: 546-554.

Nordic Committee on Foods Analysis (NMKL). (2004). Coliforms Determination in Foods Method No. 44, 6th edition.

Nordic Committee on Foods Analysis (NMKL). (2005). E. coli Determination in Foods. Method No. 125.

Nordic Committee on Foods Analysis (NMKL). (2006). Aerobic Plate Count in Foods. Method No. 86.

Nordic Committee on Foods Analysis (NMKL). (2009). Staphylococcus aureus counts in Foods. Method No. 66, 4th Ed.

Nordic Committee on Foods Analysis (NMKL). (2010). Bacillus cereus counts in Foods. Method No. 67.

Odu Ngozi Nma and Okomuda Mary Oruese. (2013). Prevalence of Salmonella species and Escherichia coli in fresh Cabbage and Lettuce sold in Port Harcourt Metropolis, Nigeria. Rep Opinion, 5(3):1-8.

Sagoo, S.K., Little, C.L., Griffith, C.J. Mitchell, R.T. (2003). Study of the cleaning standards and practices in food premises in the United Kingdom. Commun Dis Pub Hlth6, 6-17.

Stenfors Arnesen,L.P., Fagerlund,A., Granum,P.E. (2008). From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiol. Rev. 32, 579-606.

Tambekar, D.H. and Mundhada, R.H. (2006). Bacteriological quality of salad vegetables sold in Amravati city (India). Journal of Biological Science 6: 28-30.

Uzeh, R.E., Alade, F.A. and Bankole, M. (2009) The microbial quality of pre-packed mixed vegetable salad in some retail outlets in Lagos Nigeria. African Journal of Food Science, **3**, 270-272.

Table 1. Other bacteria isolated from cabbages from the various markets

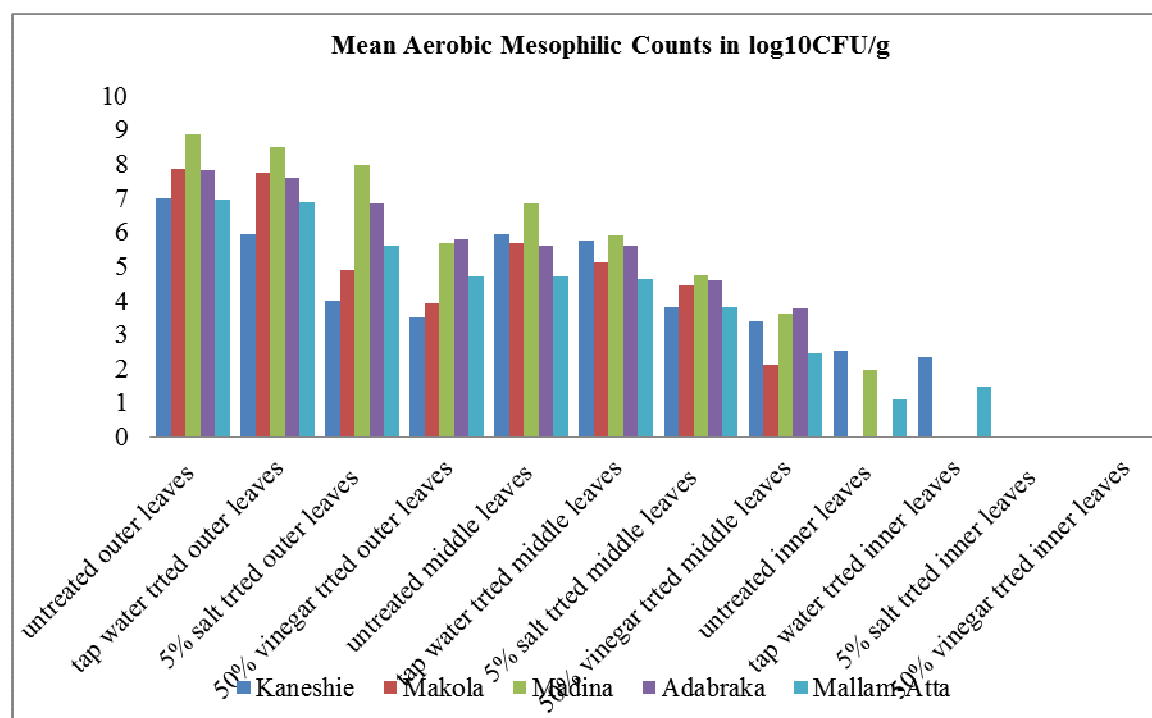| Markets	Bacteria Isolate
Kaneshie	Bacillus cereus
Makola	Bacillus cereus, Staphylococcus aureus
Madina	Bacillus cereus, Staphylococcus aureus
Adabraka	Bacillus cereus
Mallam-Atta	Bacillus cereus, Staphylococcus aureus

Figure 1: Mean population of aerobic mesophiles from five different market centre's before and after treatment with tap water, 5 % salt solution or 50 % vinegar solution.
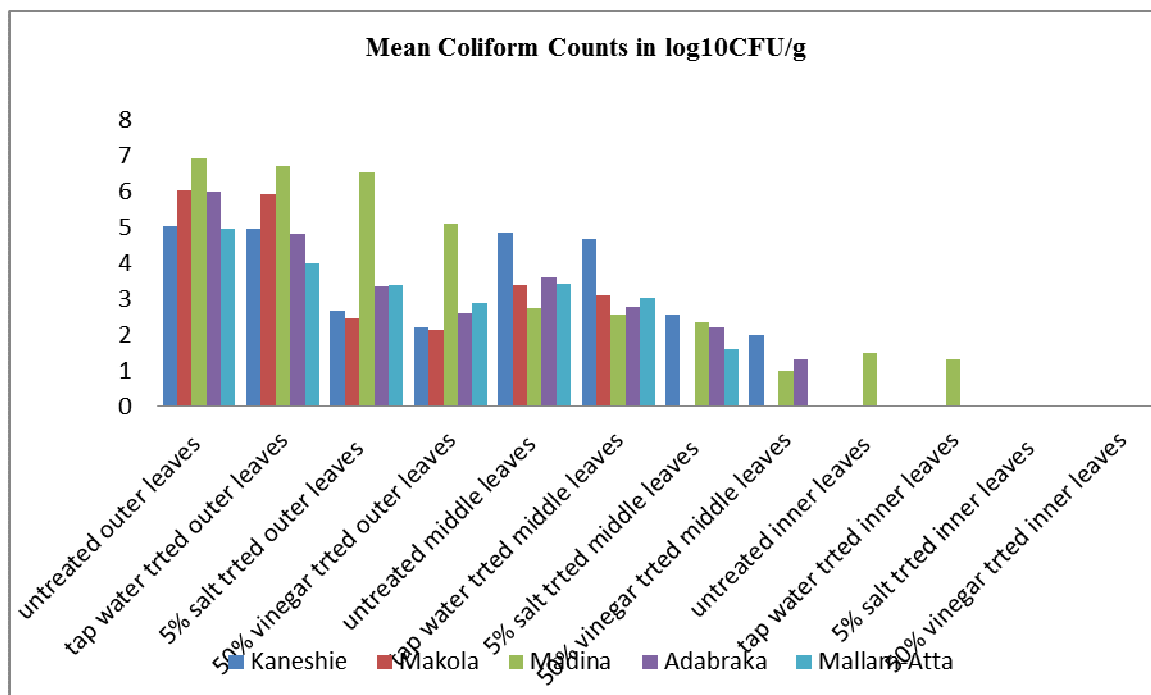
Figure 2: Mean population of coliforms from five different market centre's before and after treatment with tap water, 5 % salt solution or 50 % vinegar solution.
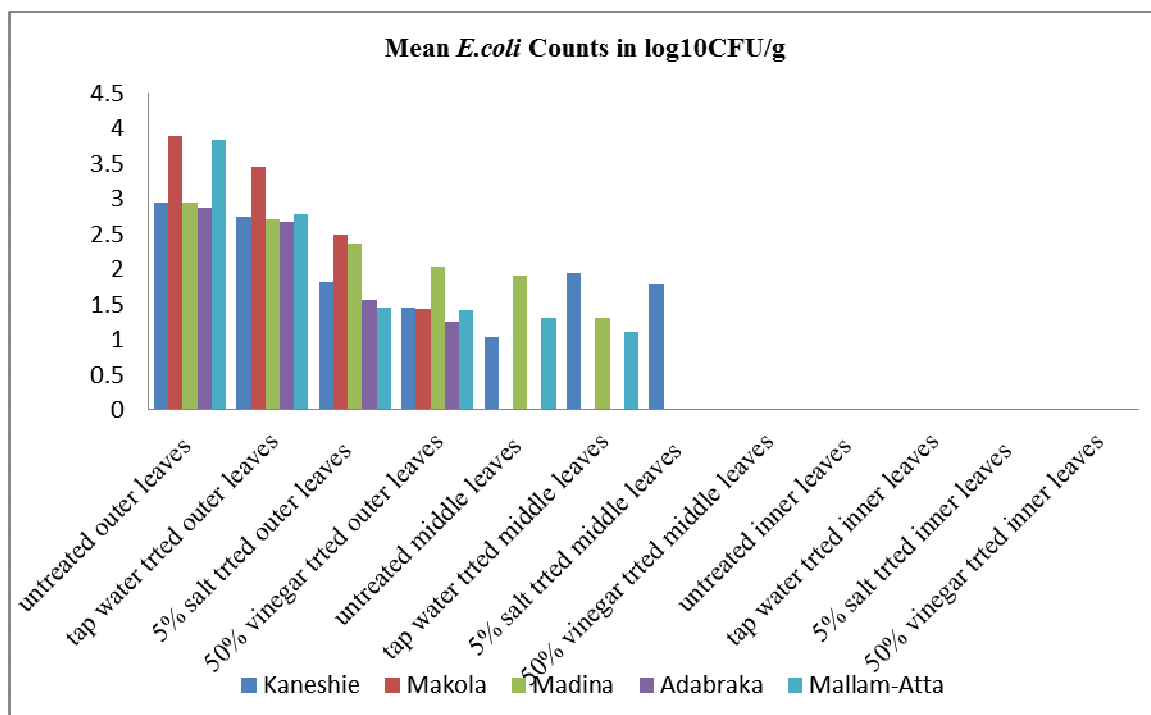

Figure 3: Mean population of E. coli from five different market centre's before and after treatment with tap water, 5 % salt solution or 50 % vinegar solution.

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