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Effect of washing agents on the bacterial quality of lettuce and cabbage sold in wukari market Taraba state, Nigeria.

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Abstract

Vegetables while growing may be exposed to many sources of contamination like contaminated sewage water used in watering the gardens from where these vegetables are grown. This study was conducted to determine the general bacterial quality of lettuce and cabbage and the effects of washing agents at different contact time so as to establish its microbiological safety. Cabbage and lettuce vegetables were weighed (5grams) each and washed in 250 ml solutions of vinegar, sodium chloride, sterile distilled water, and potable water. The volume of vinegar and quantity of sodium chloride used was 2 ml, 4 ml and 7 ml/g and 2g, 4g and 7g/ ml at < 1 minute, 2 minutes and 5 minutes contact time respectively. In the case of sterile distilled water, and potable water only contact time of 5 minutes was assessed. The results of the total aerobic plate count of bacterial cells ranged from 3.0x107 to 9.2x107cfu/ ml in lettuce and 3.2x107 to 6.9x107cfu/ ml in cabbage. Effect of different contact time for lettuce and cabbage showed some coliform counts for samples treated with sodium chloride at concentrations of (2g) in less than 1 minute and potable water at 5 minutes (4.3x107, 3.0x107, and 4.7x107 cfu/ ml) respectively. However, the samples treated with vinegar, sodium chloride and sterile distilled water at various concentrations of 4 and 7 ml with a contact time of 2 and 5 minutes showed zero coliform count. The following bacteria were isolated from the vegetable samples; Proteus vulgarius, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, Staphylococcus aureus, and *Pseudomonas aeruginosa* which could be due to natural flora in the soil or contaminants. The findings of this study revealed the efficiency of vinegar which had a significant effect on the microbial load reduction amongst the various washing agents used.in relation to the different contact time. Therefore, lettuce and cabbage should be washed in solutions of vinegar / sodium chloride so as to ensure safety of the consumers.

Keywords: Bacterial quality; Cabbage; Lettuce; Washing agents; Concentrations; Contact time

1. Introduction

A vegetable is a soft and herbaceous parts of plant that may be eaten raw or cooked alone or in combination with other products like meat, fish, and other preparations [1]. Vegetables are known to be rich in vitamins, iron, calcium, proteins, fats and minerals. Leafy green and yellow vegetables are valued highly for their vitamin A and iron contents. Vegetables are helpful in neutralizing the acid substances produced in the course of digestion of meat, cheese and other foods as they are valued as roughage's which promotes digestion and helps to prevent constipation [2]. While growing, vegetables may be exposed to many sources of contamination like contaminated sewage used in watering the gardens from where these vegetables are grown. *Salmonella s*pecies and other pathogens are derived from raw and treated sewage [2].

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Consumption of leafy greens have recently increased because of the demand for nutritious foods that ensures sufficient intake of vitamins, minerals, fibers and phenolic compounds with antioxidant and antimicrobial properties [3]. Leafy greens are routinely consumed as salads, appetizers, spices, or even just as a garnish, and often eaten raw without any heat treatment [4]. In spite of their nutritional values, conventional leafy greens are generally exposed to microbial contamination through many points such as cultivation, harvesting, storage, and marketing in the production system [5]. Consequently, there is a corresponding likelihood that consumers are prone to infections by enteric pathogens [6]. Hot climate and environmental conditions (such as dusty streets crowded by vehicles) favor bacterial growth in leafy greens sold either by street vendors or at retail local markets. Organic foods is a small but a growing part of the food industry and its existence provides an element of consumer choice being safer [7] and respectful to the environment[8]. There is a concern that they may contain higher levels of pathogenic bacteria than conventional produce, due to increased use of manure rather than chemical fertilizers [9]. The presence of *E. coli* (normally not pathogenic to humans) in fresh produce not only reflects fecal contamination [10], but also indicates a possibility that other potential pathogens associated with fecal contamination could be ingested and capable of causing infections [7].

In recent years, there has been an increasing demand for fresh-cut vegetables [11]. Concomitant, fresh-cut or ready-to-eat produce including raw produce are becoming more popular [12, 13]. Since 1994 the global production of vegetables increased 62% from 566 247 tons to 908 838 tons in 2008 [14]. This increase is due to the nutritional value and the assumed beneficial health effects. Well-known diseases like cancers and heart diseases can be avoided through regular consumption of fruit and leafy vegetables such as lettuce, spinach and cabbage or in one-word fresh produce [15].

Several reports showed that enteric diseases linked to consumption of fresh produce have dramatically increased in the last decades [16,17,18]. For this reason, fresh produce is recognized as a potential vehicle for transmission of pathogenic microorganisms known to cause human disease. Outbreaks associated with fresh produce result in economic losses to farmers, distributors and the food industry [19].To reduce and/or eliminate the microbiological contamination of vegetables after harvest, proper washing of fruits and vegetables is essential for decontamination [20]. Only clean potable water should be used for irrigation, the drinking water should be used for bulk-soil removal and first cleaning [21]

Vigorous washing in potable water typically reduces the number of microorganisms by 1–2 logs and is often as effective as treatment with 100 ppm chlorinated water, the current industry standard [22]). Water supplemented with varying concentrations of organic acids, such as citric and ascorbic, acetic acids (vinegar) and sodium chloride has been shown to reduce microbial populations on fruits and vegetables [20]. Washing and sanitizing agents can reduce the levels of surface contamination of raw and processed fresh produce items, and therefore, can help reduce the likelihood that large focal outbreaks may be associated with specific contamination events [23].

A worrying trend that has emerged in recent years is the growing number of food borne diseases that have been linked to green salad vegetables, especially lettuce and cabbage despite its nutritional value and this has been of concern as one could not stand to tell if the washing agents has effect on the vegetables as food contamination may occur at any point during its journey through production, processing, distribution and preparation [24]. It is anticipated that the findings from this study will enhance the suitability of the use of different washing agents so as to establish the microbiological safety of salad vegetables (fresh produced.) to the consumers. .

2. Material and methods

2.1. Sample Collection

Samples of salad vegetables, cabbage and lettuce were purchased from New Market Wukari, Taraba State, Nigeria. The vegetables were packaged in ice packed containers and transported to Food Science and Technology Department of Federal University Wukari.

2.2. Sample Preparation

The method as described by [25] was adopted with slight modification. Five (5) grams each of cabbage and lettuce vegetables were weighed and washed in 250 ml of each solution of vinegar, sodium chloride solution, sterile distilled water, and potable water by vigorous shaking. Effect of different contact time of vegetables and wash solutions, and different concentrations of wash solutions were determined. The volume of vinegar used was 2 ml, 4 ml and 7 ml/g and the quantity of sodium chloride used was 2g, 4g and 7g/ ml at < 1 minute, 2 minutes and 5 minutes contact time. In the case of sterile distilled water, and unsterilized (potable water) only contact time of 5 minutes was assessed.

2.3. Preparation of Media

All media were prepared according to manufacturer's instructions, and sterilized by autoclaving at 121^{0}C for 15minutes. Plate count agar was used for the determination of total aerobic plate count and MacConkey Agar was used for the isolation of coliforms.

2.4. Isolation and Enumeration of Microorganisms

Total bacterial count was determined using the method of [26] for Samples of lettuce and cabbage . The stock solution was prepared by taking 1 ml of the washed sample of lettuce and cabbage and mixing with 9 ml of sterile peptone water. Each sample was serially diluted (10 fold dilutions $10^{-1,}$ $10^{-2,}$) in sterile peptone water and 0.1 ml amount of the dilution 10^{-2} were spread on various agar media; Plate count agar and MacConkey agar and incubated at "37 ^{o}C " for 24hrs for total aerobic and coliform count respectively, developed colonies were counted. .All enumerations were expressed as colony forming units per milliliter (cfu/ ml) of plated samples. Pure colonies of bacteria were maintained on slants of medium of isolation and stored at 4^{o}C until when needed.

2.5. Identification and Characterization of Isolates

Bacterial isolates were identified based on their microscopic (Gram staining reaction), morphological, cultural, and biochemical tests (catalase,indole oxidase,coagulase,citrate and appropriate sugars for characterization using standard methods [27], and [28]. The pure bacteria isolates were identified as described by the method of [9].

3. Results and discussion

The effect of washing agents in different concentrations and time on the bacterial quality of lettuce and cabbage sold in Wukari, Market were studied. Table 1a and b. showed microbial load of lettuce and cabbage for the total aerobic plate count of bacterial cells which ranged from 3.0 x107 cfu/ ml to 9.2x107 and 3.2x107 to 6.9x107 cfu/ ml for lettuce and cabbage respectively. Among the two vegetables (lettuce and cabbage) analyzed, lettuce had the highest bacteria load of 9.2x107 cfu/ ml (Table 1a and b). The high bacterial load in lettuce can be linked to its structure of its leaves having a large surface area suitable for water contact making them likely to be affected by bacterial contamination.

Tables 2a and b showed the results for coliform count of the bacterial cells. This study revealed a low coliform count of 3.0 x 107 and 4.3x107 cfu/ ml when compared with samples of lettuce treated with sodium chloride at concentration of " 2 g "and potable water respectively. Other washing agent's vinegar and sterile distilled water at concentration of 2, 4 and "7 ml " showed zero coliform count. However, the result of this study revealed the efficiency of the various washing agents and this result is contrary to that of [29] who reported an average count for total coliform and faecal coliform of 5.95x104 and 6.13x102 cfu/ ml for lettuce respectively. This improvement could be as a result of the different washing agent's vinegar, sodium chloride and sterile distilled water employed in this study.

The samples washed at higher concentration of washing agents had less bacterial load than those washed with only water. Vinegar proved to be more effective than sodium chloride and water on the bacterial load of lettuce and cabbage which might be due to the acidic pH of vinegar as some microorganisms cannot withstand acidic conditions. This research revealed the efficiency of washing lettuce and cabbage in relation to time. It was observed that the microbial load decreased with increase in contact time from <1 – 5 minutes for all the three concentrations used. The more the contact time and/or concentration, the greater the exposure period of the more vulnerable bacteria to the unfavorable wash solutions and the effect on the bacterial cell. The result of this study is agreement with the report of [20] who also obtained a similar result in his study. Sodium chloride also had an effect on the bacterial load though at a more reduced rate than that of vinegar solution. Not all microorganisms can withstand salt or saline conditions and this could be a reason for its effect on the bacterial load. Sterilized water had effect on the bacterial count, but not as much as vinegar and sodium chloride solution. This might be because fewer bacteria may have adhered to the lettuce and cabbage after washing with sterilized water. However, the sterilized water is a more favorable medium for the organisms than sodium chloride solution and vinegar. This might be because sterilized water (neutral pH) had no inhibitory effect as in the cases of vinegar and sodium chloride solutions on the bacteria present on the lettuce and cabbage. Potable water however had the least effect on the total bacterial and coliform count. Washing lettuce and cabbage with different washing agents led to reduction of the bacterial count especially vinegar and sodium chloride.

Figure1.0 showed the different bacteria isolated and their prevalence from the various vegetable samples: lettuce and cabbage. The isolates include the following; *Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus* *subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus and Staphylococcus epidermidis*, which could be due to natural flora in the soil or contaminants. The prevalence of the organisms isolated are as follows *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (5.26%), *Bacillus subtilis* and *Escherichia coli* (10.52%), *Salmonella typhi* and Staphylococcus aureus (15.78%) and *Staphylococcus epidermidis* (26.31%) respectively. *Staphylococcus epidermidis* had the highest prevalence of occurrence (26.31%) (Fig 1.0). *Proteus vulgaris* isolated from lettuce a rod shaped, nitrate-reducing gram-negative bacteria present in the soil from where they can get in contact with vegetables, if consumed on vegetables can cause urinary tract infections, The presence of *Staphylococcus epidermidis* and Staphylococcus aureus in the vegetables which may have been introduced by handlers since it is part of the natural flora, Bacillus species are spore formers and are everywhere in nature. They can contaminate vegetables especially through spores present in the environment, they are also part of the natural flora and its presence can cause diarrhea, abdominal cramps, vomiting and nausea, the presence of *Escherichia coli* in the samples analyzed can be attributed to irrigation water containing untreated sewage or manure, The presence of other pathogens such as *Salmonella typhi* in the vegetables may have been introduced through irrigation with contaminated water which when consumed can cause typhoid fever, nausea, blood in stool and diarrhea, The detection of *Enterobacter aerogenes* and *Pseudomonas aeruginosa* which are present in the soil can also contaminate vegetables when they get in contact with the vegetables. The microorganisms present in fruits and vegetables can be a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage and processing of the produce [30].

Table 1a Total aerobic plate count (TAPC) of bacterial cells (cfu/ ml) on lettuce

Vegetable				
Sample code	**Treatment (reagents)**	**Quantity of reagents used**	**Time (Minutes)**	**TAPC (cfu/ ml)**
C1	Vinegar (ml/g)	2 ml	<1	4.2 $x10^7$
C2	„	4 ml	2	3.6 $x10^7$
33	„	7 ml	5	3.0 $x10^7$
C4	NaCl(g/ ml)	2g	<1	$8.7x10^7$
C5	„	4g	2	$8.2x10^7$
C6	„	7g	5	$6.3x10^7$
C7	Sterile water distilled water	7 ml	5	$4.2x10^7$
C8	Portable water	7 ml	5	$9.2x10^7$

cfu/ ml = Colony forming unit per millitre of sample; Nacl = Sodium Chloride

Table 1b Total aerobic plate count (TAPC) of bacterial cells (cfu/ ml) on cabbage

Vegetable				
Sample code	**Treatment (reagents)**	**Quantity of reagents used**	**Time (Minutes)**	**TAPC (cfu/ ml)**
D1	Vinegar (ml/g)	2 ml	<1	$3.1x10^7$
D2	„	4 ml	2	NG
D3	„	7 ml	5	NG
D4	NaCl(g/ ml)	2g	<1	$6.9x10^7$
D5	„	4g	2	$3.2x10^7$
D6	„	7g	5	NG
D7	Sterile distilled water	7 ml	5	$3.2x10^7$
D8	Portable water	7 ml	5	$4.3x10^7$

CFU/ ml = Colony forming unit per millitre of sample; Nacl = Sodium Chloride ; NG = No growth.

Table 2a Total aerobic plate count of bacterial cells (cfu/ ml) of lettuce (coliform count)

Sample code	Treatment (reagents)	Quantity of reagents used	Time (minutes)	Coliform counts (CFU/ ml)
A1	**Vinegar (ml/g)**	2 ml	<1mins	NG
A2	"	4 ml	2mins	NG
A3	"	7 ml	5mins	NG
	Nacl (g/ ml)			
A4	"	2g	<1min	$4.3x10^7$
A5	"	4g	2mins	NG
A6	"	7g	5mins	NG
A7	Sterile distilled water	7 ml	5mins	NG
A8	Potable water	7 ml	5mins	$3.0x10^7$

Table 2b Total aerobic plate count of bacterial cells (cfu/ ml) of cabbage (coliform count)

Sample code	Treatments (reagents)	Quantity of reagents used	Time	Coliform Count
B1	**Vinegar "**	2 ml	1 min	$4.5x10^7$
B2	"	4 ml	2 mins	NG
B3	"	7 ml	5 mins	NG
	Nacl (g/ ml)			
B4	"	2 g	<1 min	$3.1x10^7$
B5	"	4 g	2 mins	NG
B6	"	7 g	5 mins	NG
B7	Sterile distilled water	7 ml	5 mins	NG
B8	Potable water	7 ml	5 mins	$4.7x10^7$

KEY; A1 – A3= vinegar, A4 –A6= sodium chloride, A7=sterile distilled waterA8= potable water, B1–B3=vinegar, B4–B6= sodium chloride, B7=sterile distilled water, B8=potable water, NG =no growth

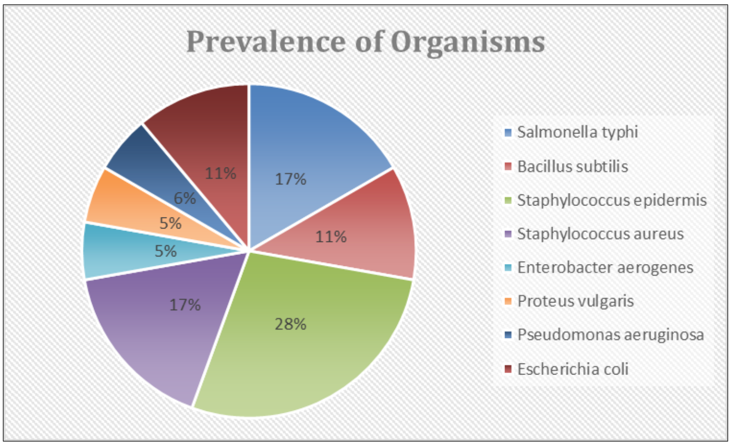

Figure 1 Bacteria Isolated from Lettuce and Cabbage Washed with Different Washing Agents and their Prevalence

4. Conclusion

The findings of this study revealed the efficiency of the various washing agents on lettuce and cabbage. Vinegar had the highest effect on the bacterial load of vegetables. This could be due to its acidic pH in which some microorganisms cannot withstand acidic conditions. It could also be coined that increase in contact time from <1 –5 minutes also had a significant effect as the microbial load decreased from $9.2x10^7$to $3.0 x10^7$ cfu/ ml in lettuce and $6.9x10^7$ to $3.2x10^7$ cfu/ ml in cabbage. Washing lettuce and cabbage with different washing agents could therefore be seen as having a reduction on the bacterial load. Therefore, lettuce and cabbage should be properly washed especially in solutions of vinegar and sodium chloride and rinsed properly before consumption.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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