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Microbial Quality of Selected Ready-to-Eat Vegetables from Iwo, Nigeria and Effectiveness of Rinsing Agents

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ABSTRACT

Consumption of fresh vegetables promotes good health and is encouraged but possess a wide range of microbial contaminants and have been implicated in several foodborne disease outbreaks. Washing can reduce microbial load of pathogens thereby minimizing harm to consumers. The focus of this study was to assess the effectiveness of rinse agents (plain water, salt, vinegar and chlorine solutions), on the microbial content of ready- to- eat vegetable (cucumber, spinach and lettuce) samples using standard methods. Microbial load of unwashed vegetables ranged from (3.8×10^5 - 1.2×10^7 CFU/g); (1.2×10^6- 2.1×10^7 CFU/g); and (1.3×10^7 - 2.6×10^7 CFU/g), while microbial load for washed vegetables ranged from ($5.0 \pm 0.7 \times 10^4$ - $5.8 \pm .7 \times 10^6$ CFU/g); ($2.8 \pm 0.7 \times 10^5$ - $1.9 \pm 0.0 \times 10^7$ CFU/g); ($3.8 \pm 0.7 \times 10^5$ - $9.8 \pm 0.7 \times 10^6$ CFU/g) for total viable, enterobacteriaceae and staphylococcal count respectively. There was 1-2 log reduction in the microbial load of vegetables after rinsing. Chlorine and vinegar solutions were more effective than plain water. Nine genera of bacteria were identified before rinsing including Staphylococcus, Pseudomonas, Bacillus and E. coli. However, after rinsing, 2 to 7 species were identified. This study showed that ready to eat vegetables are contaminated with different bacteria genera of public health concern. Plain water was not effective enough for the reduction of microbial load therefore chlorine or vinegar rinse could be adopted.

Key words: Ready-to-eat vegetables, salad, rinse agents, chlorine, vinegar

INTRODUCTION

Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw or cooked (Fayemi, 1999; Dhellot *et al*., 2006). They contain valuable nutrients which can be successfully utilized to build up and repair the body. Examples of the nutrients include vitamins such as vitamins A, C, K and B6, as well as pro-vitamins, dietary minerals protein, fat and carbohydrates (Woodruff, 1995, Whitaker, 2001).

Traditionally, vegetables are cooked and consumed along with meat or fish dish. However, the demand for raw, ready-to-eat, fresh-cut vegetables has continuously increased (Jacxsens *et al.,* 2010) because of the high nutrient levels. For example, vitamin C content is higher in raw fruits and vegetables compared to the cooked. This nutrient is heat labile and lost in cooking water while raw vegetables are generally low in calories.

Vegetables such as Lettuce *(Lactuca sativa*), cucumber (*Cucumis sativus* L.) and spinach (*Spinacia oleracea*) are common vegetables popularly consumed raw around the world including Nigeria (Brackett, 2010). Cucumber (*Cucumis sativus* L.) is a creeping vine which bears cylindrical edible fruit when mature. It has a high water content making it a diuretic and also has a cleansing action within the body. Daily consumption of cucumber juice helps in curing various digestive disorders and acts as a drug against the problem of constipation (Vora *et al.,* 2014). Spinach (*Spinacia oleracea*) is an edible flowering plant in the family *Chenopodiaceous*. There are two types of regularly consumed spinach – smooth leaf and Savoy leaf. Lettuce *(Lactuca sativa*) is a member of the *Asteraceae* family, with high water content, providing magnesium, vitamin C and beta-carotene. These selected vegetables have a widespread use in salads and sandwiches while supporting healthy diet (Mathieu and Diouf, 2007). They are very good source of dietary fibre, manganese, potassium, biotin, copper iron, omega-3 fatty acids and pantothenic acid (Ensminger, 1996).

Differences in microbial profiles of various vegetables result largely from unrelated factors such as resident microflora in the soil, introduction of non-resident microflora via animal manures, sewage, irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007, Ofor *et al*., 2009). In developing countries such as Nigeria, continuous use of untreated waste water and manure used for irrigation and fertilizers, respectively for the production of fruits and vegetables are the major contributing factors of microbial contamination to these plant products (Amoah *et al*., 2009). Furthermore, these factors have been documented to cause foodborne disease outbreaks in developed and developing countries (Nascimento, 2003). Center for Disease and Control (2016) defined foodborne disease outbreak as the occurrence of at least two cases of similar illness, resulting from the consumption of common food. Causative agents for foodborne disease include, but not limited to bacteria, virus, fungi and prions present in foods.

Fresh-cut vegetables are highly susceptible to microbial cross-contamination through the shredders and slicers or cutting knives (Garg *et al.,* 1990), leading to the exposure of inner tissues to microbial attachment and growth after cutting (Brackett, 1992). Since these vegetables are considered ready-to-eat and consumed raw, the possibility of food poisoning therefore exists (Aycicek *et al*., 2006). Foodborne disease outbreaks could be due to several microorganisms such as *Staphylococcus aureus, Salmonella Enteritidis, Campylobacter jejuni, Clostridium perfringes, Bacillus, Shigella sonnei, Escherichia coli O157:H7, Listeria monocytogenes and Thermotolerant coliform* (CDC, 1997, Beuchat, 1996, Farber and Brown, 1990).

Vegetable vendors generally do not follow good hygienic practices (CDC, 2009; Dun-Dery and Addo, 2016). For instance, vendors who do not maintain good personal hygiene can carry microorganism on their skin, hair, hands or cloths and may unintentionally contaminate fresh fruits or vegetables, thus creating the opportunity to transmit foodborne pathogen (CDC, 2009). The presence of these foodborne pathogens in ready-to-eat foods suggest that the prevalence and vulnerability of foodborne diseases and deaths from consuming these foods will be high in Nigeria (Ajayi and Oluwoye, 2015).

Washing and disinfection have been considered the only operations aimed actively at reducing microbial populations on fresh cut vegetables and for this reason they are important stages in food safety for raw vegetable consumption (Nascimentos, 2003). Chlorine solutions such as calcium hypochlorite or other antibacterial compound including vinegar, salt solution have also been employed to reduce the number of contaminating microorganisms in vegetables (Lund, 1983), due to the ineffectiveness of only tap water in removing microorganism. Use of chlorine have been documented to inactivate or destroy pathogenic bacteria, fungi, viruses and creates about 1 to 2 log reductions on fresh vegetables (Allende *et al.*, 2008). Vinegar/acetic acid is inexpensive and an effective disinfectant active against bacteria. In fact, acetic acid has been used as an alternative to chlorine for surface decontamination of vegetables (Sengun and Karapinar, 2004).

Many consumers who rely extensively on ready-to-eat meals, travel long distances to work and desire to eat balanced diets. Vegetables such as lettuce, cucumbers and spinach are sold on the street and in open markets and generally consumed raw without further or thorough washing. However, most vegetables are exposed to several mishandling processes from harvesting to sales in open market thereby increasing the microbial load. In order to reduce the number of gastrointestinal infections as a result of consuming raw vegetables, there is a need to investigate the washing agents most suitable for reducing the microbial load of the selected ready-to-eat vegetables.

MATERIALS AND METHODS

Materials

Selected ready-to-eat vegetables mainly lettuce *(Lactuca sativa L*), cucumber (*Cucumis sativus* L.) and spinach (*Spinacia oleracea*) were purchased from several vendors in open markets in Odo-Ori and Oluwo, Iwo, Nigeria. All the samples were collected in sterile universal containers and plastic bags and transported to the Food Science and Technology Laboratory (Bowen University, Iwo, Nigeria) for processing immediately. Disinfecting agents such as chlorine powder (Zenith Lab. Ikeja, Nig.), acetic acid (vinegar) (Ritebrand LTD. UK.) and Table salt (Royal Salt Ltd., Lagos, Nigeria) were also procured.

Preparation of Rinse Agents

About 0.3 g of chlorine was diluted in 2 L of water to give 100ppm and pH of 7.1 (Sapers, 2001). Vinegar and water solution was mixed in a 1:1 (v/v) (Eni *et al.,* 2010), and 1 g of salt was mixed in 2 L of water (w/v) (Nascimento *et al*., 2003). These rinse agents were prepared and used immediately.

Washing

Approximately 500 g of each vegetable sample was washed thoroughly in the different rinse agents namely chlorine; vinegar; salt solution and plain potable water for 5 mins and subsequently rinsed in clean water for 30 s to remove residual chemical. A set of vegetable sample was not subjected to any washing and was used as control or baseline of the microbial load of the vegetables.

Quantification of Bacteria

Ten grams of each vegetable type after rinsing was added to 90 mL of peptone water, and further serially diluted up to 10^{-4}. Nutrient agar (NA), Mannitol salt Agar (MSA) and MacConkey Agar (MAC) (Park scientific London, UK) were inoculated with 0.5 mL of the last dilution in duplicates using the pour plate method for total viable, Staphylococcal and Enterobacteriaceae counts respectively. The plates were incubated at 37 ^{0}C overnight and enumerated after incubation.

Identification of Isolates

Following enumeration, morphological characteristic of each colony, elevation, size and colony margin were observed. Distinct colony was sub-cultured to achieve pure culture for further biochemical studies (indole, Methyl Red (MR), Voges Proskauer (VP), catalase, carbohydrate fermentation etc.) and identification of organisms (Pollack *et al.,* 2002).

Statistical Analysis

Data collected from tests were analysed using Statistical Package for the Social Sciences (SPSS) (2011). Analysis of Variance (ANOVA) was used to evaluate significant differences and separation of the mean values was carried out using Duncan Multiple Range Test at ($p<0.05$).

RESULTS AND DISCUSSION

Microbial Analysis

The microbial quality of vegetables as well as the effect of different rinse agents on the microbial load was investigated and reported in Table 1. All the agar plates had observable growths, indicating that all the vegetable samples in this study were heavily contaminated. The unwashed samples (control) had the highest total viable (TVC), Enterobacteriaceae and Staphylococcal counts and significantly ($p<0.05$) different from washed vegetables.

Cucumber had the highest TVC and enterobacteriaceae count compared to spinach and lettuce. For instance, TVC of the vegetables before application of rinse agents were $1.2\pm7.0\times10^7$, $1.6\pm0.7\times10^6$ and $3.8\pm0.7\times10^5$ CFU/g for cucumber, spinach and lettuce, respectively. Enterobacteriaceae counts enumerated were $2.1\pm7.0\times10^7$, $2.3\pm0.14\times10^6$ and $1.2\pm7.0\times10^6$ CFU/g cucumber, spinach and lettuce, respectively. Staphylococcal counts were $1.3\pm7.0\times10^7$ and $2.6\pm7.0\times10^7$ CFU/g for cucumber and spinach, respectively were reported before rinsing (Table 1). After rinsing, the microbial load of the vegetables varied with the different rinse agent.

Overall, after rinsing, chlorine and vinegar solution were more effective in reducing total viable, Staphylococcal and enterobacteriaceae counts. Cucumber and Spinach had 1-Log reduction in TVC after chlorine rinsing ($1.8\pm0.7\times10^6$ and $3.2\pm0.0\times10^5$ CFU/g, respectively). Vinegar and chlorine rinse also resulted in a 1-Log reduction in enterobacteriaceae count for lettuce ($3.8\pm0.7\times10^5$ CFU/g) and spinach ($2.8\pm0.7\times10^5$ CFU/g). There was 2-Log reduction for Staphylococcal count in Spinach ($6.2\pm0.7\times10^5$ CFU/g) with chlorine rinse.

The microorganisms present in vegetables are a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce (Beuchat, 1996; Ray and Bhunia, 2007). The results of high microbial loads for selected ready-to-eat vegetables from Iwo, Osun State concurs with other researchers who also reported high content of bacteria (Abdullahi and Abdulkareem, 2010; Itohan *et al.*, 2011; Atter *et al.*, 2014).

The results of the effect of chemical treatments on microbial load of vegetables revealed that each of the chemical reduced microbial count to various degrees. Chlorine reduced the microbial count more than vinegar and salt solution. Shalaby and El-Raliman, (1995) reported that salt solution was not effective against some bacteria. However, it was more effective in this study than plain water and reduced Staphylococcal count by 1-2 logs when used for cucumber and spinach washing (Table 1). Chlorine and vinegar are acidic in nature and are bactericidal (Entani, *et al.*, 1998), while salt is alkaline and bacteriostatic in nature (Ostergaard, 1994). In terms of toxicity, chlorine, vinegar and salt solution in the concentration used in this study is generally recognized as safe (GRAS) (Frazier and Westhoff, 1998). It can be deduced from this study that rinsing vegetables in appropriate concentration of chlorine and vinegar for at least five minutes will help to reduce the level of microorganisms

Identification of isolates

Six species of microorganisms were found on cucumber and four from spinach and lettuce, respectively as presented in Table 2. In the unwashed (control) vegetables, the following organisms were found *Staphylococcus aureus, Pseudomonas, Bacillus* spp.*, Proteus* spp.*, Streptococcus, Enterobacter aerogenes, Micrococcus* spp.*, Lactobacillus* and *E. coli* (Table 2). *E. coli and Enterobacter aerogenes* are generally present in sewage, faeces, soil and water, which commonly come in contact with vegetables. Notably, *Staphylococcus aureus* was present in all the vegetable samples.

The *Staphylococcus aureus* isolated was an indication of poor hygienic practices by both the farmers and handlers. *Pseudomonas* spp. *and Bacillus* spp. are part of plant microflora and are among the most common vegetable spoilage bacteria (Vanderzant, 1992), though some *Bacillus species* are capable of causing food borne illness (Bhunia, 2008). *Staphylococcus aureus* is a pathogenic organism and of public health concern and it was isolated in most of the samples as well as other pathogenic and opportunistic bacteria such as *E. coli*, *Pseudomonas* and *Proteus*. This further highlights the need to safeguard the health of the consumers by proper washing and decontamination of these

Table 1: Mean Microbial load (CFU/g) of selected vegetables before and after washing in various rinse agents

Microbial load (CFU/g)/Vegetable	Rinse Agent				
	Unwashed (Control)	Tap water	Salt water	Vinegar water	Chlorine water
TVC[3]					
Cucumber	$1.2\pm7.0^{a}\times10^{7}$	$5.8\pm.7^{b}\times10^{6}$	$2.3\pm21^{d}\times10^{6}$	$2.8\pm.7^{c}\times10^{6}$	$1.8\pm0.7^{e}\times10^{6}$
Spinach	$1.6\pm0.7^{a}\times10^{6}$	$1.2\pm.7^{b}\times10^{6}$	$1.1\pm.14^{c}\times10^{6}$	$5.2\pm0.7^{d}\times10^{5}$	$3.2\pm0.0^{e}\times10^{5}$
Lettuce	$3.8\pm0.7^{a}\times10^{5}$	$2.8\pm0.7^{b}\times10^{5}$	$1.9\pm7.0^{c}\times10^{5}$	$5.0\pm0.7^{d}\times10^{4}$	$1.8\pm0.7^{d}\times10^{5}$
Enterobacteriaceae					
Cucumber	$2.1\pm7.0^{a}\times10^{7}$	$1.9\pm0.0^{b}\times10^{7}$	$1.8\pm7.0^{c}\times10^{7}$	$5.4\pm21^{e}\times10^{6}$	$1.2\pm7.0^{d}\times10^{7}$
Spinach	$2.3\pm.14^{a}\times10^{6}$	$2.1\pm7.0^{b}\times10^{6}$	$3.6\pm0.7^{d}\times10^{5}$	$4.0\pm7.0^{c}\times10^{5}$	$2.8\pm0.7^{e}\times10^{5}$
Lettuce	$1.2\pm7.0^{a}\times10^{6}$	$5.6\pm6.0^{a}\times10^{6}$	$9.3\pm0.0^{b}\times10^{5}$	$3.8\pm0.7^{b}\times10^{5}$	$5.1\pm0.7^{b}\times10^{5}$
Staphylococcal count					
Cucumber	$1.3\pm7.0^{a}\times10^{7}$	$9.8\pm0.7^{b}\times10^{6}$	$4.5\pm7.0^{c}\times10^{6}$	$1.0\pm0.0^{e}\times10^{6}$	$3.2\pm0.7^{d}\times10^{6}$
Spinach	$2.6\pm7.0^{a}\times10^{7}$	$2.3\pm7.0^{b}\times10^{7}$	$8.8\pm0.0^{d}\times10^{5}$	$1.1\pm0.7^{c}\times10^{7}$	$6.2\pm0.7^{e}\times10^{5}$
Lettuce	ND*	$1.0\pm0.0^{a}\times10^{6}$	$9.3\pm.14^{b}\times10^{5}$	$3.8\pm0.7^{d}\times10^{5}$	$5.1\pm10^{c}\times10^{5}$

Values are mean ± SD of duplicates; Values with different letters in same row are statistically different ($p<0.05$) using Duncan mean separation; TVC = Total viable count; ND* = Not determined

Table 2: Biochemical reactions of isolates from selected vegetables

Type of vegetable	Gram Rxn	Indole	MR	VP	Citrate	Motility	Catalase	Starch Hydrolysis	Glu	Mann	Lac	Probable organism
Cucumber												
1	+C	-	-	-	+	-	+	+	A/-	A/-	A/-	*Micrococcus* spp.
2	+C	+	+	-	-	+	+	+	A/-	A/G	A/-	*Streptococcus* spp.
3	+R	-	+	-	+	+	+	-	A/-	-	A/G	*Lactobacillus* spp.
4	+C	-	+	+	+	+	+	+	A/-	A/-	A/-	*Enterobacter* spp.
5	-R	-	+	-	+	+	+	+	A/-	-	-	*Pseudomonas* spp.
6	+C	+	-	-	+	-	+	+	A/G	A/G	A/G	*S. aureus*
Spinach												
1	-C	-	+	+	+	+	+	+	A/-	A/-	A/-	*Enterobacter* spp.
2	+R	-	+	-	+	+	+	+	A/-	-	-	*Pseudomonas* spp.
3	+C	+	-	-	+	-	+	-	A/G	A/G	A/G	*S. aureus*
4	-R	-	+	-	-	+	-	-	A/G	-	-	*Proteus* spp.
Lettuce												
1	-R	+	-	-	+	+	+	-	A/G	A/G	A/G	*E. coli*
2	+R	-	-	-	-	+	+	-	A/G	A/G	A/G	*Bacillus* spp.
3	+C	+	-	-	+	-	-	-	A/G	A/-	A/G	*Staphylococcus*
4	-R	-	-	-	+	+	-	-	A/G	-	A/G	*Proteus* spp.

Rxn= Reaction, MR=Methyl Red, VP=Voges Proskauer, + =positive, - =negative, C=Cocci, R=Rod, A=Acid, G=Gas

produce, which are consumed without heat treatments. The specie and frequency of organisms' isolation from the RTE vegetable samples are presented in Figure 1. Unwashed ready-to-eat vegetables had nine bacteria genera, while washing reduced the number and type of organisms isolated. The results presented in Figure 2 showed that, after rinsing with chlorine solution, *Proteus* spp. was the only bacteria isolated from lettuce while *Enterobacter aerogenes, proteus and Staphylococcus aureus* were still present in all the three vegetables after rinsing with vinegar.

Table 3: Two-way analysis of variance and interaction between type of rinse agent and vegetable

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Type of vegetable	1.31371E+14	2	6.56853E+13	166939.761	0
Type of rinse agent	6.27417E+13	4	1.56854E+13	39864.599	0
Type of veg * Type of rinse agent	8.76775E+13	8	1.09597E+13	27854.116	0
Error	5902010000	15	393467333.3		

Staphylococcus and Bacillus organisms were isolated from two vegetables rinsed with salt solution. *Micrococcus, Enterobacter aerogenes, Streptococcus, Proteus and Lactobacillus* organisms were isolated from the three vegetables rinsed with tap water. Microbial diversity reduction thus, followed this trend *viz:* unwashed vegetables > potable water > salt solution > vinegar solution > chlorine solution (Figure 2).

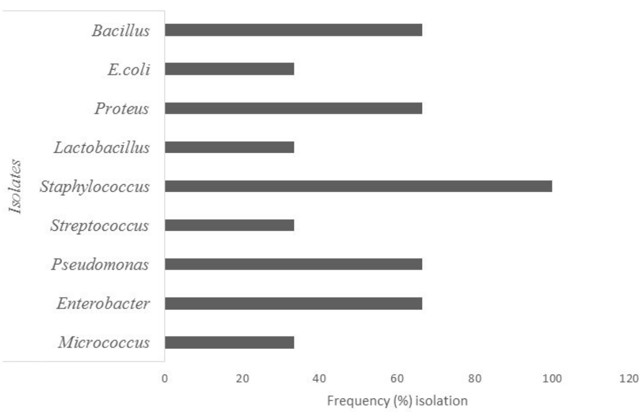

Figure 1: Frequency of bacterial isolation from unwashed selected (Cucumber, Spinach and Lettuce) vegetables

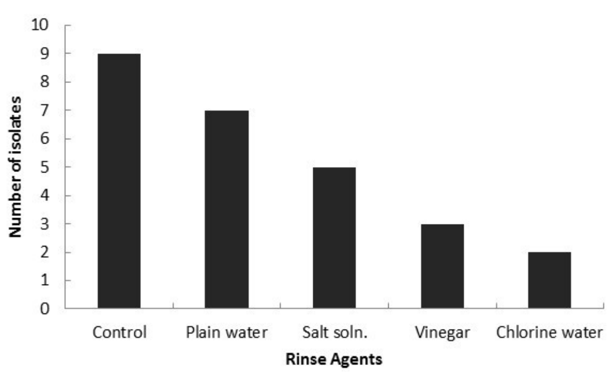

Figure 2: Effects of rinse agents on isolates from three vegetables (Cucumber, Lettuce and Spinach).

The results from this study correspond with past studies that reported that vinegar was more effective than salt water, and tap water was least effective (Atter *et al.,* 2014). Also, chlorine based sanitizer was more effective than the afore-mentioned rinsing agents when adequate doses are used (Gil *et al*., 2009). The effectiveness of the type of rinse agent and type of vegetable were further subjected to analysis of variance (ANOVA). All effects were statistically significant ($p<0.01$) (Table 3), whereas interaction effect (Type of vegetable x Treatment) is found to be statistically significant F (8, 15) = 27854.116, p=0.000.

CONCLUSION

This study has shown that the vegetables are heavily contaminated with microorganisms, which could be of public health importance. The rinse agents reduce the microbial load on vegetables by 1-2 log and the diversity of bacteria. Chlorine solution was more effective at reducing the microbial load even at low concentration (GRAS) followed by vinegar then salt solution. Good sanitary measures should be adopted while handling fruits and vegetables to limit the level of microbial contamination. Furthermore, thorough rinsing with chlorine and vinegar at low treatment levels (GRAS) can reduce the microflora of vegetables and have the potential to reduce the incidence of foodborne disease when ready-to-eat vegetables are consumed.

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