

Full Length Research paper

# Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria

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Fresh fruits and vegetables promote good health but harbour a wide range of microbial contaminants. To assess the microbial quality of fruits and vegetables sold in Sango-Ota, 15 samples of different fruits and vegetables were purchased from three vendors. Samples were analyzed to study the density of microorganisms by standard plate count (SPC). Mean microbial load ranged from  $1.3 \times 10^5$  -  $1.82 \times 10^7$  cfu/ml for vendor A;  $9.9 \times 10^6$  -  $3.0 \times 10^7$  cfu/ml for vendor B and  $9 \times 10^5$  -  $3.0 \times 10^7$  cfu/ml for vendor C. Nine bacteria belonging to eight genera were identified. *Staphylococcus aureus* (29.2%) was the most frequently isolated followed by *Staphylococcus* spp (12.5%), *Klebsiella* spp (12.5%) and *Salmonella* spp (12.5%). *Actinomyces* (4.2%) and *Escherichia coli* (4.2%) were the least frequently isolated. The effect of acetic acid (vinegar) concentration (0.5 - 2.5%) and exposure time (0-10 min) on the microbial load of five vegetables were also assessed. Increasing vinegar concentration from 0.5 - 2.5% reduced microbial loads by 15 - 82%. Least microbial loads for all vegetables were obtained when exposed to 2.5% vinegar solution for 10 min. Consumer's awareness on the dangers of consuming pathogen contaminated foods and the need to insist on properly processed/stored sliced produce needs to be re-awakened.

**Key words:** Fruits, vegetables, microbiological quality, foodborne pathogens, vinegar.

## INTRODUCTION

Fruits and vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and are thus vital for health and well being. Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006).

Fruits and vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbour a diverse range of microorganisms including plant and human pathogens (Nguyen-the and Carlin, 1994; Dunn et al., 1995; Carmo et al., 2004). Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident microflora in the soil, application of non-resident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007; Ofor et al., 2009). In

developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contamination (Olayemi, 1997; Amoah et al., 2009).

Thus despite their nutritional and health benefits, outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables have increased in recent years (Hedberg, 1994; Altekruze and Swerdlow, 1996; Beuchat, 1996, Beuchat, 2002). Enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns during food-related outbreaks (Buck et al., 2003). Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage (Beuchat, 1998). These increases in fruits and vegetables-borne infections may have resulted from increased consumption of contaminated fruits and vegetables outside the home as most people spend long hours outside the home. In Nigeria for instance, street vending of handy ready-to-eat sliced fruit and vegetables has recently become very common and the market is thriving.

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Bacteriologically safe fruits and vegetables are essential to maximize the health benefits promised by adequate consumption of these produce. Proper washing of fruits and vegetables is essential for decontamination. Water supplemented with varying concentrations of organic acids, such as acetic, citric and sorbic acids, has been shown to reduce microbial populations on fruits and vegetables (Karapinar and Gonul 1992; Beuchat, 1998). Previous studies revealed that a vinegar dip resulted in a 3 to 6 log<sub>10</sub> decrease in the number of aerobic bacteria on parsley leaves, depending on vinegar concentration used and incubation time (Beuchat, 1998).

This study was undertaken to determine the microbial load of fruits and vegetables sold on Sango, Ota, Nigeria and to determine the effect of various concentrations of vinegar (acetic acid) on the microbial load of some vegetables.

## MATERIALS AND METHODS

### Sample collection

A total of 15 samples comprising three types of fresh fruits {Apple (*Malus domestica* Borkh), Pineapple (*Ananas comosus* L.) and Water Melon (*Citrullus lanatus* (Thunb.))} and seven types of salad vegetables {Cabbage (*Brassica oleracea* L.), Carrot (*Daucus carota* L.), Cucumber (*Cucumis sativus* L.), Green Pepper (*Capsicum annuum* L.), Lettuce (*Lactuca sativa* L.), Runner beans (*Phaseolus coccineus* L.), and Spring Onions (*Allium fistulosum* L.)} were collected from 3 different sources; a road side vendor, a local market and a cafeteria in Sango-Ota. Three of the fruits samples (two pineapples and one water melon) were sliced ready to eat fruits. All the samples were collected in sterile universal containers and plastic bags and transported to the laboratory for processing.

### Isolation of microorganisms

Nutrient agar, MacConkey Agar, Sabrouad-Dextrose agar, Salmonella-Shigella agar, peptone water (all from Fluka, Germany), Urea agar base, tryptone broth (both from Lab M, UK), mannitol salt agar (Oxoid, England), Methyl red voges-proskauer broth (Scharlau, Spain) and Simmons-citrate agar (Biomark, India) were prepared according to Manufacturer's instruction, and sterilized by autoclaving at 121°C for 15 min. Salmonella-Shigella agar, which does not require autoclaving, was sterilized by boiling for 15 min.

Twenty-five grams of each sample was weighed and washed in 100 ml of sterile distilled water. MacConkey, *Salmonella-Shigella* agar and nutrient agar were inoculated with 1 ml of the rinse water using the Pour Plate Technique. The plates were allowed to solidify, inverted and incubated at 37°C for 24 h for colony formation. Each colony was isolated in a pure form by sub-culturing for further studies and identification. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification was based on the methods of Jolt et al. (1994).

### Microbial Load determination

For enumeration of microorganisms present in each sample, 10-fold serial dilutions of each rinse water were made and 1 ml of 10<sup>-2</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> dilutions were pipetted into sterile Petri-dishes and molten nutrient agar (45°C) was added and swirled thoroughly to allow

even distribution. The colonies were counted using a colony counter (Stuart Scientific, UK) after 24 h incubation at 37°C.

### Effect of (acetic acid) vinegar concentration and exposure time on microbial load

To determine the effect of varying concentrations of acetic acid solution on the microbial load of vegetables, five vegetables (Cabbage, Carrot, Cucumber, Green Pepper and Lettuce) were purchased from a local vendor and 25 g of each sample was weighed and washed in 100 ml of 0.5, 1.5 or 2.5% acetic acid (vinegar) solutions. Aliquot of 0.1 ml of each rinse solution was inoculated on nutrient agar at the initial time of rinsing, then after 5 and 10 min exposure. Number of colonies on each plate was counted using a colony counter. Normal household vinegar contains about 5% acetic acid. A concentration of 2.5% acetic acid was obtained by making a 1:1 dilution of household vinegar with distilled water and subsequently diluted accordingly to obtain other dilutions.

## RESULTS

### Microbial load of the fruits and vegetable samples

All the fruits and vegetables sampled in this study were contaminated. However, the microbial load of the fruits and vegetables varied with type and vendor (Table 1). Microbial load ranged from 1.3 × 10<sup>6</sup> - 1.82 × 10<sup>7</sup> cfu/ml for vendor A; 9.9 × 10<sup>6</sup> - 3.0 × 10<sup>7</sup> cfu/ml for vendor B and 9 × 10<sup>5</sup> - 3.0 × 10<sup>7</sup> cfu/ml for vendor C (Table 1). Apple from vendor C had the lowest microbial load (9 × 10<sup>5</sup> cfu/ml) of all the fruits and vegetables sampled, while spring onions from vendor B and pineapple from vendor C had the highest microbial load (3.0 × 10<sup>7</sup> cfu/ml). Table 1 also shows that of the three ready to eat sliced fruits sampled in this study, pineapple from vendor A had the least microbial load (1.3 × 10<sup>6</sup> cfu/ml) while pineapple from vendor C had the highest microbial load (3.0 × 10<sup>7</sup> cfu/ml).

### Organisms isolated from the fruit and vegetable samples

Based on cultural, morphological and biochemical characteristics of the organisms isolated, a total of nine bacteria were identified and number of the different bacteria isolated from each of the samples varied (Table 2). *S. aureus* (29.2%) was the most frequently isolated being present in 7 of the 10 fruit and vegetable types sampled followed by *Staphylococcus* spp (12.5%), *Klebsiella* spp (12.5%) and *Salmonella* spp (12.5%). *Actinomycetes* (4.2%) and *E. coli* (4.2%) were the least frequently isolated (Table 2). *Salmonella* spp was present in all the fruits sampled but not in any of the vegetables while *Staphylococcus* spp, *Micrococcus* spp, *Klebsiella* spp, *Pseudomonas* spp and *Actinomycetes* were isolated from the vegetables but not from any of the fruit sampled.

**Table 1.** Total plate count of 15 fruits and vegetable sampled in Sango Ota, Nigeria.

Fruit sample	Microbial load (cfu/ml)		
	Vendor A	Vendor B	Vendor C
Carrot	$3.8 \times 10^6$	$2.9 \times 10^7$	$7.8 \times 10^6$
Runner beans	$9.1 \times 10^6$	$9.9 \times 10^6$	NT
Cucumber	$1.3 \times 10^7$	NT	$4.6 \times 10^6$
Pineapple	$1.3 \times 10^6$	NT	$3.0 \times 10^7$
Green Pepper	$3.6 \times 10^6$	NT	NT
Cabbage	$1.8 \times 10^7$	NT	NT
Spring Onions	NT	$3.0 \times 10^7$	NT
Lettuce	NT	$1.7 \times 10^7$	NT
Water Melon	NT	NT	$1.0 \times 10^7$
Apple	NT	NT	$9.0 \times 10^5$

NT= Not tested.

**Table 2.** Occurrence of nine bacteria isolated from fruits and vegetables sampled in Sango Ota, Nigeria.

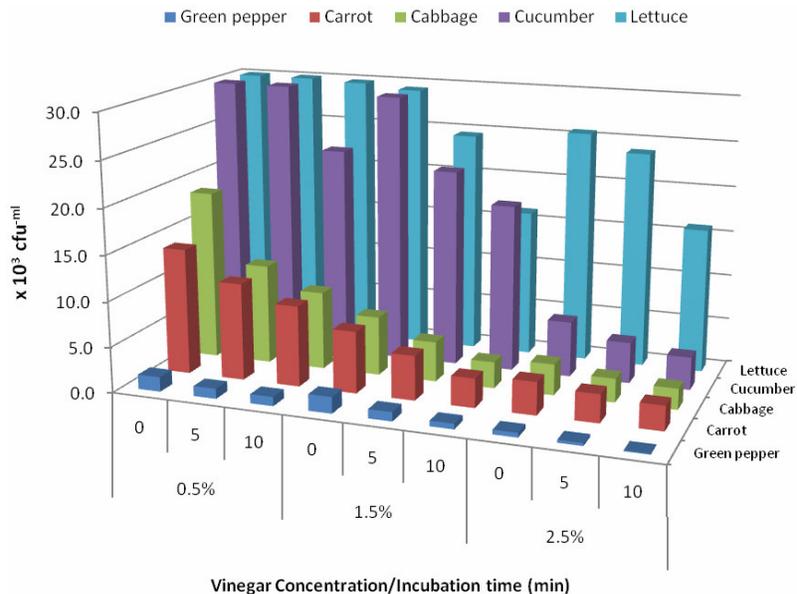
Bacterial isolates	Frequency no. (%)	Fruit samples					Vegetable samples				
		Apple	Pineapple	Water melon	Carrot	Cabbage	Cucumber	Green pepper	Lettuce	Runner beans	Spring onions
<i>Bacillus</i> spp	2 (8.3)	-	-	+	-	+	-	-	-	-	-
<i>Escherichia coli</i>	1 (4.2)	-	+	-	-	-	-	-	-	-	-
<i>Salmonella</i> spp	3 (12.5)	+	+	+	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	7 (29.2)	+	+	+	+	+	-	+	-	+	-
<i>Staphylococcus</i> spp	3 (12.5)	-	-	-	-	-	+	+	-	-	+
<i>Micrococcus</i> spp	2 (8.3)	-	-	-	+	-	-	-	+	-	-
<i>Klebsiella</i> spp	3 (12.5)	-	-	-	-	+	-	-	+	+	-
<i>Pseudomonas</i> spp	2 (8.3)	-	-	-	-	-	-	-	-	+	+
<i>Actinomycetes</i>	1 (4.2)	-	-	-	+	-	-	-	-	-	-
Total	24 (100)	2	3	3	3	3	1	2	2	3	2

### Effect of acetic acid (vinegar) concentration and exposure time on microbial load of vegetable samples

Figure 1 shows the effect of various acetic acid concentrations and exposure time on microbial

load of five vegetable. Increasing the concentration of the acetic acid (vinegar solution) used in washing from 0.5 to 2.5% resulted in 15 - 82% reduction in the microbial loads of the various vegetables (Figure 1). Highest percentage microbial load reduction due to increase in vinegar

concentration was observed in cabbage while lowest reduction was observed in lettuce. Figure 1 also clearly shows that microbial load decreased with increase in the exposure time from 0-10 min for all the three vinegar concentration used in this study. Lowest microbial load for all the vegetables



**Figure 1.** Effect of vinegar concentration (0.5-2.5%) and exposure time (0-10 min) on microbial loads of five vegetables sampled in Sango Ota, Nigeria.

were obtained when exposed to 2.5% vinegar solution for 10 min.

## DISCUSSION

The microorganisms present in fruits and 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). All the bacteria isolated in this study have previously been isolated from fruits and vegetables in other studies, both in Nigeria and elsewhere (Dunn et al., 1995; Olayemi, 1997; Adebolu and Ifesan, 2001; Omemu and Bankole, 2005; Tambekar and Mundhada, 2006; Uzeh et al., 2009). The high bacteria counts observed for the fruits and vegetables in this study are similar to those obtained in other studies in Nigeria (Uzeh et al., 2009; Bukar et al., 2010) and bacteria population as high as  $10^8$  to  $10^9$  CFU/g were reported for sprouted onion and alfalfa (Prokopowich and Blank, 1991) although lower counts have also been observed particularly for produce stored and sold in supermarkets (Pradnya and Patel, 2008).

The high microbial contamination observed in the fruits and vegetables in this study may be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling. Bacteria on storage materials may transfer to produce and cross contamination between produce is probable particularly where produce are pre-washed with the same wash water by the vendor or processor. More importantly, bacteria on the produce may multiply over time

depending on the storage conditions especially those that are psychro-trophic (Montville and Matthews, 2008; Abadias et al., 2008). Current study aimed at reporting the microbial quality of fruits and vegetables at the point of sale in stalls in Sango Ota, a suburb of Ogun State which is heavily dependant on its proximity to Lagos and to Cotonou (Benin Republic) through the border town of Idiroko for supplies of the types of fruits and vegetables sampled.

The disparity observed in the microbial load of sliced ready to eat pineapples from two different vendors is similar to the disparity observed for the microbial load of carrot from all three vendors (Table 1) and could indicate that handling by individual vendors/processors significantly affects the level of microbial contamination of fruits and vegetables.

Some of the bacteria isolated in this study may be part of the natural flora of the fruits and vegetables or contaminants from soil, irrigation water, the environment during transportation, washing/rinsing water or handling by processors (Ofor et al., 2009). *Pseudomonas* spp. and *Bacillus* spp. are part of the natural flora and are among the most common vegetable spoilage bacteria (Vanderzant and Splittstoesser, 1992), though some *Bacillus* species (*B. cereus*) are capable of causing food borne illness. The presence of *S. aureus*, a pathogenic organism of public health concern, in most of the samples and the presence of other pathogenic and opportunistic bacteria like *Salmonella* spp. and *Klebsiella* spp. in some of the fruits and vegetables, further highlights the need to safeguard the health of the consumers by proper washing and decontamination of these produce which are consumed without heat treatment.

Results of this study further confirm previous reports of microbial load reduction observed in vegetables washed/rinsed in vinegar (Karapinar and Gonul 1992; Beuchat, 1998; Amoah et al., 2009). The efficacy of the method used for microbial load reduction is usually dependent on the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of cleaner/sanitizer, pH, and temperature (Parish et al., 2003).

Increases in concentration and exposure time were found to be significant in the role of vinegar as a decontaminant for the reduction of microbial population on vegetables as observed in this study. The observed proportionate reduction in microbial loads with increase in vinegar concentration can be attributed to the further reduction in pH resulting from increased vinegar concentration. Most bacteria survive in alkaline pH better than acidic pH. Furthermore, the progressive reduction in microbial loads with increase in exposure time may be due to continuous exposure to this unfavorable pH. As vinegar treatment is likely to reduce the risk of food borne illness associated with potentially contaminated vegetables, vinegar may serve as a simple and inexpensive disinfectant for processors of sliced ready to eat vegetables in Nigeria. A possible disadvantage however, is that vinegar may change the taste of the vegetable but this can be overcome if vinegar rinse is followed by rinsing in portable drinking water.

Despite the high microbial counts obtained for some of the samples in this study, it is important to note that these samples did not show any visible signs of spoilage. Thus outward appearance may not be a good criterion for judging the microbial quality of fruits and vegetables. All fruits and vegetables should therefore be adequately washed before consumption either by the consumer or the processor and where possible, decontaminants such as vinegar should be included in the wash water.

To limit the introduction of pathogenic bacteria to vegetables through irrigation, the origin and distribution of irrigation water should be known. Where wells are used, such wells should be well-maintained, and all irrigation sources should be monitored routinely for human pathogens (Buck et al., 2003). Manure used as fertilizer should be treated either by composting or aging to eliminate pathogenic microorganisms and farmers should be educated on the need to allow sufficient amount of time between the final manure application and harvest. Fruit and vegetable processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of contamination. Processors/vendors should also observe strict hygienic measures to ensure that they do not serve as source of chance inoculation of microorganisms and contamination. There is a need to make a law compelling vendors, in Nigeria, to transport/sell sliced ready to eat fruits and vegetables in cool temperature controlled carts similar to those used for the transportation/sales of yogurts and

ice creams.

Although the number of samples studied was small and sample size varied among vendors due to unavailability of some produce, we believe this study provides a general overview of the microbiological quality of fresh-cut fruits and vegetables sold in Sango Ota, Ogun State, Nigeria.

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