

# Bacteriological Quality of Fresh Vegetables and Peeled Sugar-Cane Obtained from Selected Markets in Zaria, Nigeria

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## Abstract

Fresh vegetables and peeled sugar-cane serves as an essential component of a healthy diet, but despite their benefit they are prompt to bacterial contamination. The bacteriological quality of four types of fresh vegetables and peeled sugar-cane obtained from selected markets in Zaria, Nigeria was determined using standard microbiological method. The fresh vegetables, peeled sugar-cane, water and hand-swab samples were collected from four different locations namely; Samaru, Sabon-gari, Tudun-wada and Zaria-city. The total bacteria plate counts ranged from  $3.3 \times 10^6$  cfu/g to  $1.6 \times 10^7$  cfu/g with Tudun-wada having the highest bacteria load in lettuce. The coliform count of fresh vegetables and peeled sugar-cane varies from  $1.3 \times 10^4$  to  $8.6 \times 10^5$  cfu/g in the entire markets with Tudun-wada having the highest value in cucumber, the counts were obviously above specified Standard of  $10^5$  cfu/g and  $10^3$  cfu/g (ICMSF, 1978). Pathogenic bacteria isolated include; *Citrobacter freundii*, *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Shigella* spp., *Serratia* spp., *Cronobacter sakazaki*, *Staphylococcus aureus*, *Pseudomonas* spp., and others *Staphylococci* spp.. This study showed that, fresh vegetables and peeled sugar-cane are contaminated with pathogenic bacteria making them unsafe for human consumption; hence fresh produce should be pre- treated thoroughly, so as to reduce the risk of food- borne outbreaks.

**Keywords:** Pathogenic bacteria · Bacteriological quality, Microbiological method, Coliform counts and food-borne outbreaks.

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## INTRODUCTION

Foodborne illnesses are major public health problem which have led to an increase in morbidity and mortality worldwide (Scallan *et al.*, 2011). Globally, an estimate of 2 million people died from diarrheal diseases in 2005 and approximately, 70% of these were foodborne-poisonous related (WHO, 2011). The illnesses in about 2 million of these people were related to consumption of contaminated fresh vegetables.

Fresh vegetables and peeled sugar cane serves as an essential ingredient of a healthy diet, the consumption rate of these fresh produce and peeled sugar-cane has been increased worldwide. Fresh vegetables and peeled sugar-cane are extraordinary dietary source of nutrients, micronutrients, carbohydrates, anti-oxidants, minerals, vitamins and fibres (Prigulkar, *et al.*, 2000; Reena, *et al.*, 2012; Sararaj *et al.*, 2014) and often consumed uncooked. But despite their health benefits; these fresh vegetables and peeled sugar-cane consumption is a risk for consumers (Soltan *et al.*, 2015).

Fresh vegetables and peeled sugar-cane are so vital for human health and well-being, but despite their benefits to healthy living, fresh vegetables and peeled sugar-cane have been reported for their contamination with pathogenic bacteria such as *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp., *Salmonella typhi*, *Shigella* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Vibrio* spp., *Pseudomonas* spp., *Aeromonas* spp., and *Campylobacter coli* has been reported in different regions of the world (Bukar, *et al.*, 2010; Reena, *et al.*, 2012; Eni *et al.*, 2010; Nwankwo, *et al.*, 2013 and Denis, *et al.*, 2016).

During harvesting and transportation, fresh vegetables and peeled sugar cane may be bruised thereby, providing substrates for microorganisms present on the surface of the vegetable to grow as a resulting of plant nutrients released (Nwankwo *et al.*, 2015). Production and processing of fresh vegetables and peeled sugar-cane, probably account for the level of contamination observed. Use of questionable quality inputs including used of poor quality irrigation water and poor labourer hygiene have all been identified as possible sources of contamination (Golly *et al.*, 2016)

Fresh vegetables and peeled sugar cane consumption either raw or uncooked can be the important risk factor for the transmission of pathogens (Sararaj *et al.*, 2012). **A number of reported foodborne outbreak and chronic infections have been cause by pathogenic bacteria from fresh produce** (Eni *et al.*, 2010 and Oliveira *et al.*, 2011). **There is therefore a need to evaluate the current risk that consumers of fresh vegetables are exposed to in Zaria metropolis.**

## MATERIALS AND METHODS

A total of (160) sample of fresh vegetables (carrot, cabbage, cucumber, lettuce) and peeled Sugar cane (40), hand swabs of vegetables vendors (11), and the water used in sprinkling these fresh produce (29), were collected within April to June 2019 from open markets in Zaria City, Samaru, Tudun-wada and Sabon-Gari locations of Zaria, Kaduna State. Different markets samples were collected on separate days from distinct vendors. Within 24hours of collection, samples were transported promptly to the laboratory for analysis.

### Water and Hand-Swab sample collection

The water samples (n = 26) used for sprinkling the fresh vegetables and peeled sugar-cane were collected from the storage containers into sterile containers. During transporting the samples, sterile plastic containers were used. 10ml of each water sample were diluted in 90ml sterile normal saline. Standard Serial dilution procedure was further **carried** out using pour plate method. Eleven (11) hand-swab samples were collected from fresh produce and peeled sugar-cane vendors with sterile swab sticks moistened in 0.1% sterile peptone water, by swabbing the palms of the vendors; aseptic techniques was used to avoid contamination.

### Evaluation of this samples

To suspend the phyllosphere-associated microorganisms from the surface of cabbage, cucumber, carrot, sugar cane and lettuce, 25g of fresh vegetable and peeled sugar-cane sample was added to each conical flask containing 225 ml sterile peptone water. Sample was then agitated separately in the sterile conical flask for 2-5 min to suspend surface microbes (Seow *et al.*, [2012](#)). Hand swabs of vegetables vendors, and the water samples were analyzed.

### Culture

Standard microbiological protocol was used to determined total bacteria and total coliform count. Serial dilution was made for each samples; 1 ml of each step was inoculated into duplicates of Nutrient agar (NA) plates and MacConkey agar plates (Himedia) (Sospedra *et al.*, [2013](#)). The plates were incubated at 37 °C for 24hours (Sujeet *et al.*, [2017](#)). The colonies formed on the plates were counted using colonies counter and expressed as log<sub>10</sub> colony-forming unit/g (log cfu/g).

### Aerobic colony count of swab samples

Each 10 ml of sterile distilled water in test-tubes containing swab-stick were left standing for 30minutes. New prepared 90 ml of sterile distilled water was thoroughly mixed with 10 ml of sterile distilled water containing swab stick. 1 ml of the mixture was then transferred to a test-tube containing 9 ml of sterile distilled water, further serial dilutions was made. Exactly 0.5 ml of serial dilutions 10<sup>-2</sup> 10<sup>-3</sup> and 10<sup>-4</sup> were cultured on Nutrient Agar, Mannitol salt agar and MacConkey agar petri dishes, using pour plate method. Which were further incubated at 37°C for 24hours. The number of colonies seen counted using colonies seen were counted using a colony counter and recorded as colony forming unit per gram (cfu/ml).

### B) Biochemical tests:

This biochemical tests were used, which include catalase, coagulase, motility, indole, oxidase, citrate, malonate, methyl red, Voges Proskauer (MRVP), Triple iron sugar (TSI) for Identification and characterization of isolates.

### Microgen Identification kits

The bacterial isolates were further confirmed using standard commercially available Identification kits (Microgen ID kit).

## RESULT

**Both fresh vegetables and peeled sugar-cane contained a variety of bacterial groups.** The mean total bacterial counts and the coliform count for the fresh vegetables and peeled sugar cane are shown in (**Table 1. and 2**). Lettuce had the highest mean count of 1.6 x 10<sup>7</sup>cfu/g. The coliform count was highest in cucumber (8.6 x 10<sup>5</sup> cfu/g).

The total bacterial colony count from the hand of all the eleven (n=11) fresh produce vendors from the various locations studies ranged between 1.3 x 10<sup>4</sup> – 7.6 x 10<sup>4</sup>cfu/g as show in **table 3**.

**Table 1:** Mean of Total Bacteria Count (cfu/g) of fresh vegetables and peeled sugar cane by their location of purchase within Zaria markets.

Samples	Zaria Markets			
	Samaru	Sabo-gari	Tudu-wada	Zaria-city
Cabbage	$7.4 \times 10^6$	$6.9 \times 10^6$	$5.8 \times 10^6$	$1.2 \times 10^7$
Carrot	$1.1 \times 10^7$	$8.4 \times 10^6$	$8.4 \times 10^6$	$1.2 \times 10^7$
Cucumber	$4.8 \times 10^6$	$7.8 \times 10^6$	$1.2 \times 10^7$	$8.0 \times 10^6$
Lettuce	$1.0 \times 10^7$	$1.4 \times 10^7$	$1.6 \times 10^7$	$1.5 \times 10^7$
Sugar-cane	$3.4 \times 10^{-6}$	$3.3 \times 10^{-6}$	$1.1 \times 10^{-7}$	$7.0 \times 10^{-6}$

**Table 2:** Mean of Total Coliform Count (cfu/g) of fresh vegetables and peeled sugar cane by their location of purchase within Zaria markets.

Samples	Zaria Markets			
	Samaru	Sabo-gari	Tudu-wada	Zari-city
Cabbage	$1.1 \times 10^5$	$8.3 \times 10^5$	$3.9 \times 10^4$	$1.7 \times 10^5$
Carrot	$8.2 \times 10^4$	$1.0 \times 10^5$	$8.1 \times 10^4$	$1.2 \times 10^5$
Cucumber	$8.1 \times 10^4$	$3.2 \times 10^4$	$8.6 \times 10^5$	$6.9 \times 10^4$
Lettuce	$1.5 \times 10^5$	$1.0 \times 10^5$	$2.3 \times 10^4$	$2.7 \times 10^5$
Sugar-cane	$1.3 \times 10^4$	$2.2 \times 10^4$	$4.9 \times 10^4$	$2.1 \times 10^4$

**Table 3:** Mean of Total Bacteria count from vegetable vendors and peeled sugar-cane (hand swab) in Zaria markets

Location	AMC (log CFU/g)	TCC (Log CFU/g)
Samaru	$7.1 \times 10^4$	$1.3 \times 10^4$
Sabo-gari	$7.6 \times 10^4$	$2.8 \times 10^4$
Tudu-wada	$3.2 \times 10^4$	$2.3 \times 10^4$
Zaria-city	$3.7 \times 10^4$	$7.0 \times 10^4$

Key: AMC= Aerobic mesophilic count, TCC = Total coliform count, CFU/g = colony forming unity per grams.

Water samples obtained from the vegetable vendors were also contaminated with bacterial species with colony count ranging from  $2.0 \times 10^7 - 1.1 \times 10^8$  cfu/ml (Table 4).

**Table 4:** Mean of Total Bacteria count from water sample for rinsing the vegetables and peeled sugar cane in Zaria markets

Location	AMC (log CFU/ml)	TCC (Log CFU/ml)
Samaru	$8.5 \times 10^7$	$3.7 \times 10^7$
Sabo-gari	$1.1 \times 10^8$	$3.0 \times 10^7$
Tudu-wada	$7.0 \times 10^7$	$3.0 \times 10^7$
Zaria-city	$9.4 \times 10^7$	$2.0 \times 10^7$

Key: AMC= Aerobic mesophilic count, TCC = Total coliform count, CFU/ml = colony forming unity per millilitre.

**Tables 5: Frequency of Bacteria Isolates from all samples**

Bacteria	Frequency
<i>S. aureus</i>	45 (20.8%)
<i>Staphylococcus</i> spp.	54 (25%)
Enterobacteriaceae	92 (42.6%)
Non-Enterobacteriaceae	25 (11.6%)
<b>Total</b>	<b>216 (100%)</b>

The microorganisms isolated from fresh vegetables, peeled sugar-cane, water and hand swab samples include; *Citrobacter freundii*, *Escherichia coli*, *Enterobacter* spp., (*E. aerogene*, *E. gergovia* and *E. cloacae*) *Klebsiella* spp., (*K. oxytoca*, *K. ozaenae*, *K. pneumoniae*); *Acinetobacter* spp. (*A. baumannii*, *A. haemolyticus* and *A. Iwoffii*), *Shigella sonnei*., *Serratia* spp., (*S. liquefaciens*, *S. marcescens* and *S. Serratia rubidae*); *Cronobacter sakazaki*, *Staphylococcus aureus*, *Pseudomonas* pp. and other *Staphylococci* spp..

## DISCUSSION

Freshly consumed vegetables and peeled sugar-cane, have been the cause of food poisoning and thus a risk to the health of the consumers and the society as a whole (Gitahi *et al.*, 2012). Generally, most fresh vegetables and peeled sugar-cane sampled in this study are contaminated with various bacterial types. Bacteria count exceeded

International Commission on Microbiological Specification for Food (2006) guideline acceptable limit (100000tbc/25g). Due to unhygienic practices within the markets, this fresh produce and peeled sugar-cane are contaminated (Eni *et al.*, 2010; Oji *et al.*, 2016). The observation of high bacteria counts of this fresh vegetables and peeled sugar cane in this study are similar to those obtained in other studies in Nigeria (Abdullahi *et al.*, 2010; Oji *et al.*, 2016; Eni *et al.*, 2010) but dissimilar to the findings of (Osamwonyi *et al.*, 2013, Nwankwo *et al.*, 2015 and Uzeh *et al.*, 2009), who observed less bacteria load for bacteria compared to these study. This could be possibly due to the markets were these fresh vegetables were obtained.

Coliforms are usually indicators whose presence will normally indicate the probable presence of pathogenic organisms. Coliform count exceeded Food and agricultural organization (2008) guideline acceptable limit. The use of irrigation water on fresh vegetables and peeled sugar-cane in the respective markets has led high count in coliform, which indicates that *E. coli* can be transferred to vegetables even through irrigation water. A similar study on the bacterial quality of vegetables in Kano also showed a high count of coliform in fresh produces (Aliyu *et al.*, 2005).

Among the isolated bacterial pathogens *Enterobacter* spp, *S. aureus*, *Klebsiella* sp, *Acinetobacter* spp, *E. coli*, *Serratia* spp, *Pseudomonas* spp, *Cronobacter* spp, *Citrobacter* spp *Shigella* spp. were the predominant bacteria found to be associated with most of these fresh vegetable and peeled sugar-cane analyzed. The ability of *S. aureus* to cause a wide range of infections especially food- borne intoxication is a major public health problem (Tambekar and Mundhada, 2006, Aboh *et al.*, 2011). *Klebsiella* spp. and *E. coli* are common within the environment. The prominent inhabitant of soil and water is *Pseudomonas* spp. The organism is responsible for diseases of vegetables like angular leaf spot of cucumber (Uzeh *et al.*, 2009). The presence of these pathogens in fresh vegetable and peeled sugar-cane is a public health concern, as it has been implicated in several diseases (cardiovascular disease, chronic obstructive pulmonary disease and gastroenteritis). The condition of sales makes the fresh vegetables predisposed to contamination especially as practiced in Zaria where the source of water in the garden and in the market is questionable (Caron and Walker, 2004).

From the results obtained, this can expose the consumer to **health risk**. Therefore it is recommended that these fresh vegetables and peeled sugar-cane be thoroughly washed with clean water before consumption in order to avoid unhygienic practice.

## Conclusion

The bacterial counts and the coliform obtained were high above specified standard. The presence of these pathogens in the fresh vegetable samples could serve as an indicator for the need to promote awareness about the possible health hazards that could be due to poor handling of these fresh produces. There is therefore, the need for agencies to ensure that microbiological standards are established and practiced by fresh vegetables and peel sugar vendors for the handling distribution of fresh vegetables and preparation like washing with salt and clean water since these vegetables are highly patronized. This could help in reducing the bacterial load eventually before consumption.

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