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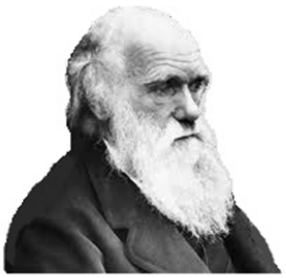

World News of Natural Sciences

An International Scientific Journal

WNOFNS 23 (2019) 43-55 EISSN 2543-5426

Bacteriological profile of cut fruits sold in Calabar Metropolis

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ABSTRACT

The increase in cases of food borne illnesses related to street vended fruit salad in developing countries is of serious public health concern. This study was, therefore, conducted on street-vended fruit salad, to determine their microbial quality. A total of twenty (20) fruit samples, comprising five each of fresh Apple (*Malus domestica* Borkh), Pineapple (*Ananas comosus* L.), Pawpaw (*Carica papaya*) and Watermelon (*Citrullus lanatus*) were collected from 3 different locations (Watt, Marian and Goldie) in Calabar and were evaluated by way of standard plate techniques, for microbial contaminants. The specific microbial genera were enumerated on appropriate selective media and identified using standard identification parameters with the aid of identification and taxonomic manuals. The results revealed that the mean total bacterial counts of all the fruit samples ranged from 1.07×10^3 to 3.4×10^3CFU/g, whereas, the mean total coliform counts ranged from 0.9×10^3 to 2.05×10^3 CFU/g, respectively. The differences in the mean total heterotrophic counts and coliform counts recorded for the fruits collected from the respective food vendors was statistically insignificant (P>0.01). Bacteria isolated include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp, *Shigella* sp, *Proteus mirabilis* and *Escherichia coli*. The presence of coliform bacteria and other microbial contaminants in numbers exceeding the recommended microbiological standards is a reflection of unwholesome product, hence the need for proper microbiological safety analysis of fruit salad prepared for human consumption. Therefore, 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.

***Keywords*:** Fruits, Microbial contaminants, Pathogens, Foodborne illness, Calabar Metropolis

(Received 09 January 2019; Accepted 25 January 2019; Date of Publication 26 January 2019)

1. INTRODUCTION

Over the years, there has been a significant increase in the consumption of sliced/ready to eat fruit because they are easily accessible, convenient, nutritious and, most especially, cheaper than the whole fruits (Nwachukwu *et al.,* 2008). Regular consumption of fruit is associated with reduced risks of cancer, cardiovascular disease (especially coronary heart disease), stroke, Alzheimer disease, cataracts, and some of the functional declines associated with aging (Liu, 2003). Raw foods, especially ready-to-eat salad vegetables, sprouts and cut fruits have been implicated in outbreaks of food borne diseases in both developed and developing countries (WHO, 1998).

Fruits are good dietary source of nutrients, micronutrients, vitamins and fiber for human; hence they are very essential for the overall well-being of man. The consumption of locally prepared mixed fruits, popularly known as fruit salad, has increased over the years in many parts of the world. 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 (Eni *et al.,* 2010). Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature (de Sousa, 2008) and are untrained in food hygiene (Barro *et al.,* 2006).

Sliced fruits commonly consumed in Nigeria include paw-paw, pineapple, watermelon, salad vegetables, cucumbers, carrots and pears. Their increased consumption, coupled with the associated risk of disease to which consumers may be exposed, is a matter of great concern. This increase in the consumption of sliced fruit has been linked with a parallel increase in food-borne illness (Estrada-Garcia *et al.,* 2004, Mensah *et al.,* 1999). Microbiological studies from many developing countries, carried out on street vended food articles have revealed a high bacteria count. *Salmonella* species, *S. aureus* and members of the family Enterobacteriaceae were common pathogens found in such food items (Bryan *et al.,* 1997; Mosupye and von Holy, 1999). Enteric pathogens such as E. coli and Salmonella are among the greatest concerns during food related outbreaks (Buck *et al.,* 2003).

Fruits are prone to microbial contamination because they are constantly in contact with soil, dust and water, and by handling at harvest or during post-harvest processing. Pathogenic microorganisms may also enter the fruits through damaged surfaces, such as punctures, wounds, cuts and splits. Such pathogens may become internalized, survive and grow within the fruit and consequently become health hazard to consumers (FDA, 1999). Salmonella sp has been reported to survive and grow rapidly on water melon held at room temperature and the level of contamination did not change when the melon was stored at refrigeration temperature (FDA, 1999). Outbreaks of listeriosis and salmonellosis have also been associated with the consumption of ready-to-eat fruit salad (Jones, 1990).

In Nigeria where street food vending is very common, there is paucity of information on the incidence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian, European and some African countries have revealed increased bacterial pathogens in fruit salad (Mahale *et al.,* 2008). In view of the health risk posed by the bacterial pathogens in fruit salad and the increasing demand for such street vended salad, the present study was undertaken to evaluate the microbiological quality of freshly prepared fruits sold in Calabar, Nigeria.

2. MATERIALS AND METHODS

2. 1. Sample collection

A total of twenty (20) samples comprising five each of fresh Apple (*Malus domestica* Borkh), Pineapple (*Ananas comosus* L.), Pawpaw (*Carica papaya*) and Water Melon (*Citrullus lanatus*) were collected from 3 different locations (Watt, Marian and Goldie) in Calabar. All the samples were collected aseptically in sterile universal containers and immediately placed in pre-cooled containers containing ice packs and then transported to the laboratory for analysis.

2. 2. Isolation and enumeration of bacteria

For the isolation and enumeration of bacteria in the samples, 10grams of each cut fruit sample was constituted in 90mls of sterile distilled water and then blended in a sterile blender after which 1ml of the homogenate was then constituted in 9ml of sterile peptone water. From there 10-fold serial dilution was performed and 0.1ml of last two dilutions (10^{-4} and 10^{-5}) were inoculated in triplicate on appropriate prepared media using pour plate technique. The plates were then incubated at 37 °C for 24-48h.

After incubation the plates were examined for the presence of discrete colonies. Colonies were counted using the colony counter and expressed as colony forming unit per gram (CFU/g) of sample homogenate. Specifically, total aerobic counts was performed on nutrient agar, while *Escherichia coli* was enumerated on Eosin methylene blue agar.

Mannitol salt agar and MacConkey agar were used to enumerate *Staphylococcus aureus* and non *E. coli* coliforms respectively, while *Salmonella-Shigella* agar was used for *Salmonella* counts after 24h pre-enrichment of sample homogenate in Selenite-F broth according to Oranusi and Olorunfemi, (2011). Characteristic discrete colonies on the different media were isolated and purified by repeated sub-culturing on the same media. Pure colonies were stored on agar slants at 4°C for further characterization.

2. 3. Purification and maintenance of isolates

Each discrete colony on a petri dish was transferred using a sterile inoculating loop into plates containing freshly prepared Nutrient agar and were incubated at 37 ºC for 24-48 hrs. After incubation, the colonial morphologies (cultural characteristics) of the isolates were recorded and compared with descriptive features contained in Holt *et al.,* (1995). The isolates were then preserved on nutrient agar slants and stored in the refrigerator at 4 ºC.

2. 4. Biochemical characterization and identification of isolates

The methods of Oranusi *et al.,* (2004) was employed for the identification of the bacteria isolates. The biochemical tests that were used to further characterize the bacteria are: catalase, methyl-red, oxidase, citrate utilization, and coagulase and indole tests. Oxidase test was also carried out on the Gram negative isolates to know if they are oxidase positive or negative. The identities of coliforms and bacteria was then confirmed using the identification aid outlined in Bergey's Manual for Determinative Bacteriology (Holt *et al*., 1994).

3. RESULTS

The total microbial load of fruits salad samples sold in Calabar is presented in Table 1. The result obtained showed varying microbial load in the fruit samples analyzed. The mean total bacterial counts of all the fruit samples ranged from 1.07×10^3 to 3.4×10^3 CFU/g. Where as, the mean total coliform counts ranged from 0.9×10^3 to 2.05×10^3 CFU/g. Meanwhile, it was observed that apple sample recorded the least microbial load.

Table 1. Mean viable counts of microorganism in fruit salad (mean x 10^3 CFU/g).

Fruit sample	THBC			TCC		
	Vendor A	Vendor B	Vendor C	Vendor A	Vendor B	Vendor C
Pawpaw	3.2 [a]	2.7[a]	3.4[a]	1.2[a]	1.6[a]	1.53[a]
Pineapple	2.3[a]	1.9[a]	2.8[a]	1.09[a]	0.9[a]	1.1[a]
Watermelon	1.4[a]	2.2[a]	2.6[a]	1.14[a]	1.3[a]	1.62[a]
Apple	1.07[a]	1.5[a]	1.8[a]	1.4[a]	1.45[a]	2.05[a]

Mean counts succeeded by alphabet "a "are not significantly different (P>0.01) from each other using ANOVA
THBC: Total heterotrophic bacteria count; TCC: Total coliform count

Table 2 shows the cultural and morphological characterization of the bacteria isolated from fruits salad samples used in this study. A total of 22 distinct colonies were morphologically characterized.

Eight of the isolates were Gram positive while the remaining 27 isolates were Gram negative, however, the cell morphology and arrangements as well as the pigmentation, consistency and elevation varies between the isolates. The isolates were either colorless, golden yellow, pink or red in color while others were colorless with black center, creamy and green.

Eight of them isolated are cocci in shape which were either in chains, clusters or tetrads while twenty-seven were found to be rod shaped either in pairs, chains or clusters. All the isolates were found to be either raised or flat in elevation.

The biochemical characteristics of the 22 bacterial isolates with the identity of the organisms are presented in Table 3. The identified isolates were found to belong to six different genera namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp, *Shigella* sp, *Proteus mirabilis* and *Escherichia coli* respectively. It was observed that *Staphylococcus aureus* had the highest rate of occurrence of 31.8%, followed by *Escherichia coli,* 18.2% while the least occurrence rate was recorded by *Shigella* spp of 9.2% as presented in Figure 1.

Table 2. Cultural and morphological characteristics of the isolates on Nutrient and MacConkey agar.

Isolate code	Gram's reaction	Cell morphology and arrangement	Pigmentation	Elevation	Consistency
C_1	+	Cocci in clusters	Golden yellow	Raised	Mucoid
C_2	_	Short rods in singles	Red	Raised	Mucoid
C_3	_	Rods in clusters	Pink	Flat	Mucoid
C_4	_	Rods in pairs	Colourless	Raised	Swarming
C_5	_	Rods in pairs	Colourless	Flat	Moist
C_6	_	Rods in pairs	Colourless	Flat	Swarming
C_7	+	Cocci in clusters	Golden yellow	Flat	Dry
C_8	_	Rods in singles	Colourless	Raised	Dry
C_9	_	Rods in clusters	Pink	Raised	Moist
C_{10}	_	Rods in pairs	Colourless	Flat	Swarming
C_{11}	_	Short rods in singles	Red	Raised	Moist
C_{12}	_	Rods in clusters	Pink	Flat	Mucoid
C_{13}	+	Cocci in clusters	Golden yellow	Raised	Moist
C_{14}	_	Rods in singles	Colourless	Raised	Dry
C_{15}	-	Rods in clusters	Pink	Raised	Mucoid
C_{16}	+	Cocci in tetrads	Creamy	Raised	Moist
C_{17}	_	Rods in pairs	Colourless with black center	Raised	Moist
C_{18}	+	Cocci in tetrads	Creamy	Flat	Dry
C_{19}	_	Short rods in singles	Red	Flat	Moist
C_{20}	_	Rods in pairs	Colourless	Raise	Moist
C_{21}	+	Cocci in tetrads	Creamy	Flat	Dry
C_{22}	_	Rods in pairs	Colourless with black center	Flat	Mucoid

Table 3. Biochemical characterization and identification of the isolates.

Isolate code	Catalase	Motility	Oxidase	Coagulase	Indole	Citrate	H_2S	MR	VP	Nitrate	Urease	Xylose	Raffinose	Glucose	Lactose	Mannose	Sucrose	Mannitol	Arabinose	Maltose	Ribose	Salicin	Cellobiose	Probable organism
C_1	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	*Staphylococcus aureus*
C_2	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	+	*Pseudomonas aeruginosa*
C_3	+	+	-	-	+	-	-	+	-	+	-	-	-	+	+	-	±	+	+	-	-	-	-	*Escherichia coli*
C_4	+	+	-	-	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	+	-	-	*Proteus mirabilis*
C_5	+	-	-	-	±	-	-	+	-	+	-	-	-	±	-	+	-	+	±	±		-	-	*Shigella* sp
C_6	+	+	-	-	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	+	-	-	*Proteus mirabilis*
C_7	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	*Staphylococcus aureus*

C_8	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	+	*Pseudomonas aeruginosa*
C_9	+	+	-	-	+	-	-	+	-	+	-	-	-	+	+	-	±	+	+	-	-	-	-	*Escherichia coli*
C_{10}	+	+	-	-	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	+	-	-	*Proteus mirabilis*
C_{11}	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	*Staphylococcus aureus*
C_{12}	+	+	-	-	+	-	-	+	-	+	-	-	-	+	+	-	±	+	+	-	-	-	-	*Escherichia coli*
C_{13}	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	*Staphylococcus aureus*
C_{14}	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	+	*Pseudomonas aeruginosa*
C_{15}	+	+	-	-	+	-	-	+	-	+	-	-	-	+	+	-	±	+	+	-	-	-	-	*Escherichia coli*

C_{16}	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	*Staphylococcus aureus*
C_{17}	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	-	+	+	-	+		-	-	*Salmonella* sp
C_{18}	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	*Staphylococcus aureus*
C_{19}	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	-	+	+	-	+		-	-	*Salmonella* sp
C_{20}	+	-	-	-	±	-	-	+	-	+	-	-	-	±	-	+	-	+	±	±		-	-	*Shigella* sp
C_{21}	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	*Staphylococcus aureus*
C_{22}	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	-	+	+	-	+		-	-	*Salmonella* sp

Key: + : positive; - : negative; ± : variable

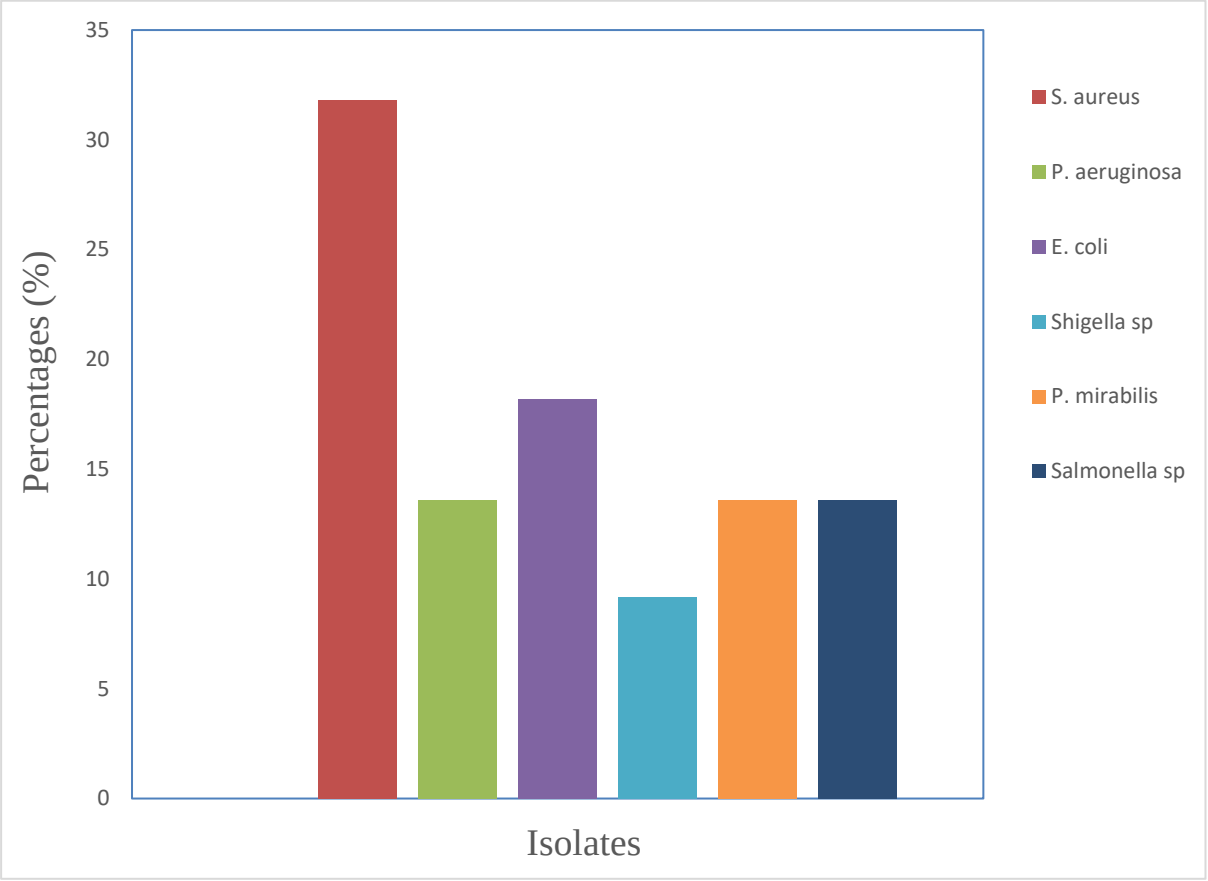

Figure 1. Percentage occurrence of the organisms isolated from the fruits samples analysed

4. DISCUSSION

The microbial loads of the fruits samples are presented on Table 1. The result obtained showed the mean total bacterial counts of all the fruit samples ranged from 1.07 x 10^3 to 3.4 x 10^3CFU/g. Whereas, the mean total coliform counts ranged from 0.9 x 10^3 to 2.05 x 10^3 CFU/g. Meanwhile, it was observed that apple sample recorded the least microbial load. These results conform to those obtained by Nwachukwu *et al.* (2008), Farzana *et al.* (2011), Oranusi and Oluwafemi (2011) who recorded microbial load in fruits in the range of 104 - 109 cfu/ml. The present investigation reveals high microbial load in the fruit salad studied. The result shows that 90% of the samples had high total viable counts ranging from 3.49 x 10^5 to 6.8 x 10^5 colony forming units per gram of the salad homogenate. The differences in the mean total heterotrophic counts and coliform counts recorded for the fruits collected from the respective food vendors was statistically insignificant (P>0.01) as presented in Table 1. The presence of these organisms in high numbers in fruit salad is of serious safety concern about the consumption of street vended foods. The high bacteria count 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 108 to 109 CFU/g were reported for sprouted onion and alfalfa

(Prokopowich and Blank, 1991). 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 was kept before they were obtained for sampling.

A total of six different bacteria species were isolated from a total of twenty (20) sliced fruits samples analyzed and they are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp, *Shigella* sp, *Proteus mirabilis* and *Escherichia coli* respectively. The isolation of these organisms is supported by the work of Eni *et al.* (2010); Jolaoso *et al.* (2010) who isolated *S. aureus, Klebsiella sp, Salmonella, Escherichia coli* from fruits. Daniyan and Ajibo (2011) also isolated *S. aureus, S. epidermidis, Bacillus sp., E. coli* and *Enterobacter aerogenes* from sliced fruits sold in Minna metropolis. This is further supported by the work of Oranusi and Olurunfemi (2011) who isolated *Bacillus, S. aureus, E. coli, Enterobacter, Salmonella, Klebsiella, P. aeruginosa, Proteus, Micrococcus* and *Lactobacillus* sp. from vended ready to eat fruits sold in Ota, Ogun State. Tambeker *et al.* (2009) also *isolated E. coli, P. aeruginosa, Salmonella, Proteus, S. aureus, Klebsiella* and *Enterobacter* from street vended fruits juices in Amravati city, India. *E. coli, K. aerogenes, P. mirabilis, S. aureus* and *Lactobacillus* were also recovered from sliced water melon (Nwachukwu *et al.,* 2008).

The incidence of the different bacteria isolated is presented on Table 6. The table showed that *S. aureus* (31.8%) and *Escherichia coli* (18.2%) are the bacteria frequently isolated from the fruits samples. Jolaoso *et al.,* (2010) and Nwachukwu *et al.,* (2008) recorded low level of *Escherichia coli* from sliced water melon. Some of the bacteria isolated in this sliced fruits may be contaminants from soil, the environment, during transportation or handling. Most of the organism isolated in this study might have been introduced into these fruits from faecally polluted water used for washing utensils (e.g. Knives, trays, and pans), wrapping material and the exposure of these products to low temperature. The presence of *S. aureus* may be explained by the fact that human beings that are processors or vendors carry this organism on/in several parts of their bodies (Nester *et al.,* 2001).

Halablab *et al.* (2011) reported that total coliform counts can be considered as a hygiene indicator especially for fecal contamination. In this study the coliform counts observed for the vegetable salads (Table 1) could suggest a high level of background fecal contamination of the vegetables used in the preparation of the commercially available salad preparations. A potential source of the contamination could be from water used in the growing and processing of the vegetable plants prior to sale. European Commission (2002) stated that cutters and slicers used in the preparation of vegetables for salad dressings can be potent sources of contamination, since they usually provide inaccessible sites, which harbor bacteria. The presence of cut surfaces provides an increased surface area for contamination and growth and allows microbial infiltration of the tissues (European Commission, 2002). Garg *et al*. (2009) and Farmer (2015) reported that exposing vegetables to various types of cutting has been shown to result in a six to seven-fold increase in microbial numbers. The presence of these coliforms especially E. coli could also be attributed to human and animal fecal contamination as these coliforms are usually present in large numbers in fecal matter.

Therefore, ordinary washing of the surfaces of fruit is not sufficient to completely eradicate microbial contaminants. Poor personal hygiene, the use of contaminated water source, inadequate washing of the component fruits and utensils and poor sanitary condition of the production area appear to be the major sources of microbial contaminants associated with contamination of fruit salad. The outcome of this study shows that street vended fruit salad pose serious health risk to consumers as they contain high level of harmful microorganisms

which may cause serious illness. Since fruit salad is usually eaten without further processing, proper processing and adoption of strict aseptic techniques and good personal hygiene should be adhered to by vendors at the preparation stage in order to reduce microbial load and eliminate microbial contamination of the final product.

5. CONCLUSIONS

This study revealed that all the fruit salads sampled from the different location within Calabar, harbored a high microbial load. Although, these microorganisms can be part of the epiphytic flora of the vegetables, their persistence and proliferation is a reflection of poor hygienic practices by both the sellers of the raw vegetables and the food handlers. The outcome of this study shows that street vended fruit salad pose serious health risk to consumers as they contain high level of harmful microorganisms which may cause serious illness. Since fruit salad is usually eaten without further processing, it is therefore recommended that proper processing and adoption of strict aseptic techniques and good personal hygiene should be adhered to by vendors at the preparation stage in order to reduce microbial load and eliminate microbial contamination of the final product. Also, clean water should be used for washing and cleaning of the utensils as well as the fruits before packaging.

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