

Microbial Safety of Polyethylene Packaged Sliced Fruits Sold in Abeokuta, South-West Nigeria

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Abstract

The microbiological quality of polyethylene packaged, sliced and ready-to-eat pawpaw, watermelon and pineapple fruits sold by street vendors in Abeokuta was assessed. Two hundred samples of RTE pawpaw, watermelon and pineapple fruits were collected from different street vendors and analyzed microbiologically. The mean aerobic plate counts ranged from 6.34 log₁₀ cfu/g to 8.99 log₁₀ cfu/g, total coliform counts ranged from 6.18 log₁₀ cfu/g to 8.43 log₁₀ cfu/g while the total fungal counts ranged from 6.18 log₁₀ cfu/g to 8.40 log₁₀ cfu/g. Bacteria isolated from these samples were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaricus*, *Bacillus* spp, *Salmonella typhi*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* while the fungi were *Penicillium* spp, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp, *Fusarium* spp, *Saccharomyces cerevisiae* and *Neurospora* spp. The sources of microbial contamination could be from the rinse water, cross contamination like knives, trays and personnel, improper handling and the wrapping materials. The presence of food-borne pathogens of microbiological standards coupled with the high total microbial counts of the samples make the fruits to be microbiologically unsafe and they should be pre- treated thoroughly before for human consumption, so as to reduce the risk of food- borne outbreaks.

Keywords: Microbiological quality, polyethylene, sliced fruits, food-borne pathogens, human consumption

1.0 Introduction

Fruits and vegetables are extraordinary dietary sources 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 as well as reducing the risk of several diseases such as atherosclerosis and cancer (Kalia and Gupta, 2006). However, fruits are known to carry non-pathogenic microflora. They become contaminated due to exposure to pathogens from various stages along its production line. Also, the increase in fruit trade among countries has increased human exposure to variety of food and fruit borne pathogens. Presence of microorganisms is a direct reflection of sanitary quality during its cultivation, harvesting, transportation, storage and processing of the produce (Beuchat, 1996; Ray and Bhemia, 2007). However, pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* spp, *Clostridium difficile*, *Campylobacter* sp, *Staphylococcus aureus*, etc have been reported with fruits and vegetables as well as the diseases resulting from consumption of the produce. Sources of pre and post harvest contamination include faeces, soil, irrigation water, improperly composted manure, air, wild and domestic animals, harvesting equipment, wash and rinse water, transport vehicles, improper storage, improper packaging, cross-contamination and improper human handling (Beuchat, 1996; Beuchat and Ryu, 1997; Afolabi and Oloyede, 2010a; Afolabi *et al.*, 2011).

In Nigeria, the consumption of sliced produce has increased in the past few years. This is because the sliced fruits are more convenient, easily accessible and most especially cheaper than whole fruits and vegetables as many consumers cannot afford the whole fruits. The fruits are peeled, cut into pieces, wrapped with transparent polythene bags and sold to the people. These fruits are sold mainly by unlicensed street vendors or hawkers with poor education levels, untrained in food hygiene and work under crude unsanitary conditions. Economic situation of the country has also contributed to the high demand of these fruits as many consumers of these produce succumb to their consumption in order to meet the nutrients requirement for their healthy living. Nevertheless, these fruits may have been contaminated with pathogenic microorganisms during processing and may be potential source of food-borne illness. Sources of contamination to these sliced fruits could be through contact with sewage and contaminated water during irrigation or rinsing. Other sources include cross contamination with knives, trays and personnel, improper handling and the wrapping materials. The consumers who depend on these fruits are more interested in their convenience than their safety, quality and hygiene.

Although, a lot of research studies have been carried out on the microbial quality of ready-to-eat foods in Nigeria, the microbial safety of sliced fruits has not been well investigated. Thus, the present study was carried out to determine the microbial safety of polyethylene packaged sliced fruits sold in Abeokuta, Ogun State.

2.0 Materials and Methods

2.1 Sample collection

In 2012, 200 samples of polyethylene packaged sliced water melon, pawpaw and pineapple were randomly collected from 50 vendors in three Local Government Areas of Ogun- State (Abeokuta South, Abeokuta North and Odeda), Nigeria. The samples were transported on ice, to the laboratory for analysis. Information on the socio-economic background, hygienic food practices and knowledge of food safety of the vendors were collected through structured questionnaires.

2.2 Microbiological analyses

Approximately 10.0g of each sample was chopped with 100.0ml of sterile peptone water (Biolab) to obtain original homogenate. Tenfold dilutions of each homogenate were then made with sterile peptone water.

Aerobic plate counts and total coliform counts were determined by plating in a standard Plate count agar (Oxoid) and MacConkey agar (Oxoid) plates in triplicates. The plates were incubated at 37°C for 48h. Colonies were counted on the plates and recorded. Pure cultures of bacterial isolates were obtained on the nutrient agar plates. Bacterial isolates were characterized on the basis of their cultural, morphological and biochemical properties and identified using Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Colonies on the MacConkey agar plates were picked and subcultured using MacConkey agar. Colonies typical to gram negative coliforms were confirmed by examining them to determine if they were non- spore forming gram negative rods that produce gas from lactose and then subjected to Indole, Methyl red, Voges- proskaur and Citrate (IMVC) tests.

Total fungal counts were determined by pour plating on Potato dextrose agar plates supplemented with lactic acid to inhibit bacterial growth, and incubated at room temperature (26± 2°C) for 72 hours. Pure cultures of the isolates were obtained by sub-culturing on the potato dextrose agar plates. Fungal isolates were characterized by their cultural properties, stained with cotton blue Lactophenol solution and observed under low power objective lens.

The prevalence of toxigenic *Staphylococcus aureus* in the samples was determined by inoculating nutrient broth supplemented with 7.5% Sodium chloride (NaCl) with the diluents. The inoculated broth was incubated at 37°C for 24h. After 24h, a loopful of culture broth was streaked onto the plates of Mannitol salt agar (Oxoid) and incubated at 37°C for 48h. The pathogenic character of the organism was ascertained by coagulase test. The haemolysis activity of the organism was determined by streaking onto blood agar and incubated at 30°C for 24h.

Enrichment of *Salmonella* and *Shigella* species was performed using alkaline peptone water. The inoculated broth was incubated at 37°C for 24h. After 24h, a loopful of culture broth was streaked onto the plates of Salmonella – Shigella agar (oxid) and Deoxycholate citrate agar (oxid) and incubated at 37°C for 48 h. Suspected colonies were subjected to series of biochemical tests.

For the detection of *Listeria monocytogenes*, 1.0ml of the diluent was spread over the plates of Blood agar and incubated at 37°C for 48h. Typical colonies were subjected to series of biochemical tests for identification.

2.3 Statistical analysis

Aerobic plate counts, total coliform counts and total fungal counts were expressed as Log₁₀cfu/g±S.D. Significant differences were established by Duncan Multiple Range test at 5% level of significance.

3.0 Results

3.1 Socio-economic background of sliced fruit vendors in Abeokuta

Table 1 shows the characteristics of polyethylene packaged sliced fruit vendors in Abeokuta. In this study, fifty sliced fruit vendors were assessed. Sliced fruits are sold in Abeokuta by 70% women and 30% men (The Hausas) aged between 20 years and >50 years. 10% of the vendors did not know their ages and the majority of them (60%) are within the age range of 30- 39 years. These Hausa men sold only sliced watermelons. 48% of these vendors are married, 6% are widows, 2% are divorced while the remaining 44% are single. Out of these fifty vendors, only 42% had primary school education, 6% had secondary school education and 52% had no formal education. None of them had either college, or polytechnic/university education. Moreover, all these vendors had never attended any course on food hygiene, but only 30% had contacted food- borne diseases before (Table 1). In addition, 42% and 45% of the vendors wash their trays and knives respectively before they use them for cutting and peeling of the fruits while 38% rinse the cut fruits before wrapping with polyethylene bags. The vendors rinse the cut fruits with well water and tap water while the remaining 12% are not rinsing their produce before wrapping. All the vendors live in bungalow buildings. The characteristics of these vendors are believed to have impacts on the microbiological quality of the sliced fruits they sell to the consumers in Abeokuta.

3.2 Microbial quality of polyethylene packaged sliced fruits sold in Abeokuta

The aerobic plate counts (APC), total coliform counts and total fungal counts of polyethylene packaged sliced fruit samples sold in Abeokuta are shown in Table 2. The fruits examined had an average APC values ranging from 6.34 log₁₀ cfu/g to 8.99 log₁₀ cfu/g. Cut-water melons had the highest APC value while pawpaw had the lowest value. Heavy bacterial contamination (>5.0 log₁₀ cfu/g) were observed in the polyethylene packaged sliced pawpaw, pineapple and water melons.

Total MacConkey agar counts (total coliform counts) of sliced fruit samples analysed ranged from 6.18 log₁₀ cfu/g to 8.43 log₁₀ cfu/g. Sliced water melon samples were also observed to contain highest number of coliform organisms and the least counts were found in pawpaw samples. In general, heavy coliforms were observed in the polyethylene package sliced fruits sold in Abeokuta (Table 2). Similarly, total fungal counts of sliced fruit samples ranged from 6.18 log₁₀ cfu/g to 8.40 log₁₀ cfu/g, with pineapple samples having the highest counts while the watermelon samples had the least counts.

In general, it was observed that polyethylene packaged sliced fruits sold in Abeokuta had unacceptable levels of contamination (>5.0 log₁₀ cfu/g for total counts and > 4.0 log₁₀ cfu/g for Enterobacteriaceae). Hence, these fruits could be adjudged as not safe for human consumption.

The incidence of *Escherichia coli*, toxigenic *Staphylococcus aureus*, *Salmonella* spp and *Listeria* spp in the sliced fruits is given in Table 3. A total of 200 samples were screened for the presence of these pathogens. All the samples were found to contain *E. coli* and toxigenic *S. aureus*. *Salmonella* spp was only found in 71 of the samples while no *Listeria* spp was isolated in any of the samples analyzed. The presence of these pathogens in these fruits indicates that the fruits are contaminated with pathogenic bacteria and are not safe for human consumption. Other bacteria isolated from the samples examined were *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaricus*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Bacillus* spp. while the fungi were *Penicillium* spp, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp, *Fusarium* spp, *Saccharomyces cerevisiae* and *Neurospora* spp. (Table 4). All these organisms are of public health significance and their presence in these produce indicates that the produce are highly contaminated and not safe for human consumption.

4.0 Discussion

Consumption of fresh fruits in Nigeria has increased as consumers strive to benefit from these products considered to be seasonal. Consumer's demand has also increased the number of fresh cut fruits available in the markets. This is because, these ready- to- eat fruits are easily accessible, convenient and cheaper than whole fruits. However, it is difficult to attest the hygiene of the processors or vendors who prepare and sell the fruits. The present study indicates that majority of the polyethylene packaged sliced water melon, pawpaw and pineapple fruits sold in Abeokuta were adjudged as 'unsatisfactory' bacterial quality as APC values and total coliform counts of the samples were higher than the recommended standard for food (<5 log₁₀ cfu of total bacterial counts per g of food sample and < 2 log₁₀ cfu of coliform per g of food sample). The quantitative results of this study support the previous findings of Thunberg *et al.*, (2002) who detected high values of APC and total coliform counts in fresh produce collected from supermarkets and markets in metropolitan Washington D. C. Also, the fruits were observed to be highly contaminated with *Escherichia coli*, toxigenic *Staphylococcus aureus* and *Salmonella typhi*, and since *E. coli* is regarded as primary indicator for microbiological quality of food and water, this shows that these fruits are not safe for human consumption. In addition, other bacterial pathogens isolated from these vegetables were *Shigella dysenteriae*, *Proteus vulgaricus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus* spp and *Enterobacter aerogenes* while the fungi were *Penicillium* spp, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp, *Fusarium* spp, *Saccharomyces cerevisiae* and *Neurospora* spp. All these bacteria are of public health significance and their presence in food indicates that such food has been contaminated with faecal materials and it is unsafe for human consumption. These results corroborate previous findings of Kaneko *et al.*, 1999; Johannessen *et al.*, 2002; Joshi and Patel, 2005 and Afolabi *et al.*, 2011. Also, the presence of fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* sp in the fruits may lead to food poisoning, since many of these fungi are toxin-producing organisms. The sources of faecal contamination to these fruits could be primarily from the irrigation water contaminated with faecal materials from abattoirs, pit latrine and human wastes (Afolabi and Oloyede, 2010a). In addition, the cut fruits may get contaminated from knives used for cutting/peeling, improper human handling and processing, tables or trays used during peeling and cutting, rinsed water, washing buckets and packaging materials as these products are cut, washed, wrapped with transparent polyethylene nylon bags and sold to the consumers.

In the present study, there was a high illiteracy rate among the fruit vendors, as majority of the vendors had no formal education, coupled with the facts that they had no knowledge of food safety and hygiene as the vendors had never attended any course on hygiene; their educational levels appeared to have impacts on the microbiological quality of the fruits they are selling.

Finally, previous study carried out by Afolabi and Oloyede (2010b) showed that high concentration of

sodium chloride solution or chlorinated water removed most pathogens on the surfaces of fresh produce. Consumers of polyethylene packaged sliced fruits are therefore advised to wash these cut-fruits thoroughly with high concentration of salt water or chlorinated water before being consumed so as to remove the pathogens present on their surfaces. This will therefore reduce the risk of food borne outbreaks in our societies.

5.0 Conclusion

In conclusion, the polyethylene packaged sliced pawpaw, pineapple and water melon fruits sold in Abeokuta are highly contaminated with food-borne pathogens, which affect their quality. However, the quality of these produce can be improved by pre- treatment of the fresh produce with various anti-microbial agents such as chlorinated water and high concentration of sodium chloride solution, so as to decrease the density of microbial contamination from the surfaces of these produce, so that the risk of food- borne pathogens decreases and chances of food- borne outbreaks can be minimized.

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Table 1: Characteristics of the polyethylene packaged sliced fruit vendors in Abeokuta, South-western Nigeria

Parameters	Frequency (%) n=50
Age (years)	
<20	0
20- 29	10(20)
30- 39	30(60)
40- 49	5(10)
>50	0
Unknown	5(10)
Sex	
Female	35(70)
Male	15(30)
Educational attainment	
Primary school	21(42)
Secondary school	3(6)
Colleges/Polytechnics/University	0
No education	26(52)
Marital status	
Single	22(44)
Married	24(48)
Widowed	3(6)
Divorced	1(2)
Years of vending experience	
1-2	14(28)
3-4	26(56)
5-6	16(12)
>6	2(4)
Type of apartment	
Bungalow	50(100)
Flat	0
Self- contained	0
Rinsing after peeling/cutting	
Yes	38(76)
No	12(24)
Type of water used for rinsing after cutting/peeling	
Well water	33(66)
Tap water	5(10)
Borehole	0
River/stream water	0
No rinsing	12(24)
Washing of trays before use	
Yes	42(84)
No	8(16)
Washing of knives before use	
Yes	45(90)
No	5(10)
Type of fruits sold	
Watermelon	33(66)
Pineapple	25(50)
Pawpaw	24(48)
Attendance of course on hygiene	
Yes	0
No	50(100)
Food-borne diseases contacted by the vendors	
Cholera	5(10)
Salmonellosis	5(10)
Enteritis	0
Dysentery	5(10)
None	35(70)

Table 2: Mean microbial counts of polyethylene packaged sliced fruits sold in Abeokuta

Fruits	Number of samples analyzed	Aerobic plate counts (Log ₁₀ cfu/g ±S.D)	Total counts (Log ₁₀ cfu/g ±S.D)	Total coliform counts (Log ₁₀ cfu/g ±S.D)	Total fungal counts (Log ₁₀ cfu/g ±S.D)
Watermelon	80	8.99 ± 1.1 ^a	8.43 ± 0.8 ^a	6.18 ± 0.1 ^a	6.18 ± 0.1 ^a
Pawpaw	60	6.34 ± 0.60 ^b	6.18 ± 0.02 ^b	6.30 ± 0.5 ^a	6.30 ± 0.5 ^a
Pineapple	60	7.25 ± 1.5 ^c	6.21 ± 0.7 ^b	8.40 ± 0.04 ^b	8.40 ± 0.04 ^b

Note: Means with different letters along the columns are significantly different (P< 0.05)

Table 3: Prevalence of *Escherichia coli*, toxigenic *Staphylococcus aureus*, *Salmonella spp* and *Listeria spp* in polyethylene packaged sliced fruits sold in Abeokuta

Fruits	Number of samples analyzed	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella spp</i>	<i>Listeria spp</i>
Watermelon	80	80	80	31	0
Pawpaw	60	60	60	18	0
Pineapple	60	60	60	22	0
Total (% incidence)	200	200 (100%)	200 (100%)	71 (35.5%)	0

Table 4: Microorganisms isolated from polyethylene packaged sliced fruits sold in Abeokuta

Fruits	Isolated microorganisms
Watermelon	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella typhii</i> , <i>Klebsiella pneumoniae</i> , <i>Shigella dysenteriae</i> , <i>Proteus vulgaricus</i> , <i>Bacillus spp</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterobacter aerogenes</i> , <i>Aspergillus niger</i> , <i>Penicillium spp</i> , <i>Fusarium spp</i> and <i>Rhizopus spp</i>
Pawpaw	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella typhii</i> , <i>Klebsiella pneumoniae</i> , <i>Bacillus spp</i> , <i>Pseudomonas aeruginosa</i> , <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Fusarium spp</i> and <i>Rhizopus spp</i>
Pineapple	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella typhii</i> , <i>Shigella dysenteriae</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter aerogenes</i> , <i>Bacillus spp</i> , <i>Pseudomonas aeruginosa</i> , <i>Saccharomyces cerevisiae</i> , <i>Neurospora spp</i> , <i>Aspergillus niger</i> , <i>Penicillium spp</i> and <i>Rhizopus spp</i>

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