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Microbiological and Nutritional Qualities of Sliced Fruits Hawked in Some Parts of Kano Metropolis, Northern Nigeria

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Abstract

This study was carried out to evaluate the microbiological quality and nutritional values of sliced fruits vended in some parts of Kano metropolis, northern Nigeria. Two hundred and forty (240) sliced fruit samples comprising of pineapple (60), pawpaw (60), water melon (60) and coconut (60) purchased at the point of sales were analyzed using standard methods for a period of six months (June to November, 2010). Aerobic mesophilic bacteria, yeasts and molds as well as coliforms were enumerated as markers of food–borne microbial contamination using standard methods. Results showed high microbial counts in all the sliced fruits analyzed from the different locations with coconut having the highest bacterial count of 3.46 x 10^5 cfu/g (site B) while pineapple had the least count of 1.70 x 10^5 cfu/g (site C). The fungal count was highest in pineapple (2.88 x 10^5 cfu/g) at site C and the least was in coconut (1.27 x 10^5 cfu/g) at site A. *E. coli*, *Staphylococcus aureus*, *Mucor* and *Aspergillus* species were isolated and characterized using cultural, morphological and biochemical methods. However, *E. coli* 0157:H7 and *Salmonella* species were not detected in all the samples analyzed. Generally, the sliced fruits were rich in carbohydrates (2.6-36.1%), indicating high nutritional quality. The ash content (1.0-2.8%) was high and could aid bowel movement and increase mineral contents respectively in the human body. High moisture (45.8-95.0%) and protein (0.4-3.6%) contents could encourage spoilage by proteophilic and hydrophilic bacteria and fungi. Poor sanitation and inadequate storage and marketing conditions may contribute to contamination and recontamination of the products. This study revealed unhygienic habit of the vendors.

***Key words*: Microbiological, Nutritional, Quality, Sliced fruits, Vendors and Food hygiene.**

Introduction

Fruits carry a natural, non-pathogenic, epiphytic microflora. However, there are certain factors, which contribute to the microbiological contamination of these products with pathogens. Poorly processed street vended produce has been identified as an important cause of deaths in developing countries (Mensah *et al*., 2002). Bacteria like the species of *Salmonella*, *Campylobacter*, *Shigella* as well as *Staphylococcus aureus* and *Escherichia coli* can contaminate sliced fruits and vegetables through contact with sewage and contaminated water (Fredlund *et al*., 1987; Blostein, 1993; Aliyu *et al*., 2005; Dahiru *et al*., 2008). Cutting and slicing removes the protective surfaces of the fruit. Cutter and slicers can be potential sources of contamination, since they usually provide inaccessible sites, which harbor microorganisms. The presence of cut surface provides an increased surface area for contamination and growth since microorganisms colonize the entire environment in which we live.

Contamination or cross contamination of street foods, especially sliced fruit and vegetables are increased by unsanitary processing as well as the open display of street food produce encourages sporadic visits by flies, cockroaches, rodents (Siame *et al*., 1996; Braide and Nwaoguikpe, 2011) and dust (Bryan *et al*., 1992).

It is difficult for one to attest to the hygiene of the processors or to the sanitary conditions at point of preparation. Moreover, the case is worsened by the fact that sliced fruits street vending is done without adequate storage conditions, thereby exposing the sliced fruits to flies and other disease causing agents. The sliced fruits are processed and sold by unlicensed vendors with poor education levels and untrained in food hygiene (Muinde and Kuria, 2005; Barro *et al*., 2007). The consumption of sliced produce may thus potentially increase the risk of food-borne diseases caused by a wide variety of pathogens. There are different sources of microbiological invasion of sliced produce. Pathogens may invade the interior surfaces of produce during peeling, slicing, trimming and other processes like packaging, handling and marketing (Barro *et al*., 2007). Preservation of sliced fruits and vegetables that requires no further processing before consumption at ambient temperature during retail maintains the produce at optimum temperatures for invasion by pathogenic mesophiles (Muinde and Kuria, 2005).

Materials and Methods

Sample collection sites

Samples of sliced fruits used for this study were purchased from different street vendors in Kano (11°-12.5°N; 8°-9.45°E). The samples were placed in a cool box at the time of sampling and immediately transported to the laboratory for analysis within 1-2 hours of collection. The sample collection sites included the following:

Site A: Kabuga market in Gwale local government area (11 59’ 13” N, 8 28’ 55” E).

Site B: Rimi market in Municipal local government area (11 59’ 48” N, 8 31’ 28” E).

Site C: Yankura market in Fagge local government area (12 00’ 44” N, 8 32’ 03” E).

Physical analysis of the samples

Determination of temperature and pH

A liquid-in-glass thermometer was used to measure the temperature of the fruit samples while pH was measured using a digital pH meter. Four random samples were selected from each of the various sites of the study for the analysis. All measurements were done immediately at the points of sample collection (Electronic Code of Federal Regulations, ECFR, 2008).

Elemental and proximate analysis of the samples

Proximate and mineral compositions of the samples were carried out according to the methods of the Association of Officials of Analytical Chemists (AOAC, 1984). The carbohydrate, protein, fats,’ ash and moisture contents of the fruit samples were determined. On the other hand, calcium, sodium, potassium, magnesium and iron were determined using flame photometer (model PFP7).

Microbiological Analysis of the Samples

Enumeration of aerobic mesophilic bacteria

A sterile knife was aseptically used to cut a portion from each fruit sample (watermelon, coconut, pawpaw and pineapple) after which 25 grams were weighed using a weighing balance and introduced into a blender. A quantity (225ml) of sterile buffered peptone water was added and aseptically blended at a speed of 15,000-20,000 rpm for 2.5 minutes. The homogenate was further mixed by shaking and 0.1ml was pipetted into a test tube containing 9.0ml of sterile buffered peptone water and diluted serially to obtain the desired appropriate dilutions (10^{-1}–10^{-3}). Here, 0.1ml of each dilution of the homogenate was pipetted and introduced into each of the correspondingly -labeled Petri dishes in duplicates. A quantity (15ml) of nutrient agar was poured into each Petri dish within 15 minutes of the time of original dilution. The sample dilution and nutrient agar medium were mixed thoroughly by swirling and allowed to solidify. The inoculated plates were invertedly incubated at 37 ± 0.5^{0}C for 24-48 hours after which all plates with 30-300 colonies were counted, recorded and expressed as colony forming units per gram (cfu/g) of the fruit sample analyzed (FAO, 1979).

Enumeration of coliform bacteria

The preparation of the homogenate and dilution were done as described for the aerobic mesopilic bacteria and the coliform counts determined using standard plate count and most probable number (MPN) techniques respectively (FAO, 1979).

Each of the three test tubes containing 9.0ml of lauryl sulphate trypose (LST) broth (each with inverted Durham tubes) was inoculated with 1.0ml of food homogenate. The same operation was carried out from the first to the second and third dilution tubes using a new sterile pipette in each case.

On the other hand, a loopful from each gas-positive tube of LST was transferred into a separate tube of brilliant green lactose bile (BGLB) broth (each containing a Durham tube). The BGLB tubes were incubated at 37±0.5^{0}C for 24-48hr. The formation of gas confirmed the presence of coliform bacteria and number of gas-positive tubes were noted, recorded and expressed as the most probable number per gram of the fruit sample (MPN/g) with reference to MPN index table.

Enumeration of yeasts and moulds

Malt extract agar (containing 100 g/l chloramphenicol) was used for the enumeration of yeasts and moulds. The preparation of the homogenate and dilution were done as described for the aerobic mesopilic bacteria. Here, 1.0ml of each dilution was aseptically pipetted and introduced into each of the correspondingly-labeled Petri dishes. A quantity (15ml) of the malt extract agar medium (tempered to 45^{0}C) was poured into each of the labeled Petri dishes and allowed to cool to room temperature. After solidification, the plates were invertedly incubated at 20-25±0.5°C for 5-7 days. The counting was reported as yeast and mould count per gram of the fruit sample analysed (FAO, 1979).

Isolation, Identification and Characterization of the Microbial Isolates

Cultural and morphological characterization of the fungal isolates

This was carried out using cultural and morphological techniques. The macroscopic and microscopic characteristics were examined. The macroscopic features were based on the physical appearance of the colonies while the microscopic features were observed under microscope using the ×40 objective lens (FAO, 1979).

Cultural, morphological and biochemical characterization of the bacterial isolates

Escherichia coli

This was carried out according to the procedure specified by the Food and Agriculture Organization (1979). Here, a loopful from each gas-positive LST test tube (previously used in the estimation of coliform count) was inoculated into a tube of EC broth and incubated for 24-48 hours at 45.5±0.5^{0}C. The initially clear broth turned turbid with gas formation. This was further streaked onto L-EMB agar and incubated at 45.5±0.5°C for 24-48 hours. The appearance of green metallic sheen colonies confirmed the presence of *E. coli*. Gram's staining of the suspected colony (*E. coli*) was prepared and observed microscopically. The appearance of Gram-negative bacilli was positive for *E. coli*. In addition, motility and biochemical (IMViC) tests were carried out to confirm the identity or otherwise of the *E. coli* (Fawole and Oso, 2001).

Escherichia coli 0157:H7

Two to three of the suspected colonies from L-EMB plates were streaked onto the sorbitol- MacConkey agar (SMAC) plates in duplicates. The plates were incubated for 24hrs at 37^{0}C and observed for colonies that appeared white, which was indicative of non-sorbitol fermenters (Chesebrough, 2000).

Staphylococcus aureus

The preparation of food homogenate and dilution were done as described for aerobic mesophilic bacterial enumeration (FAO, 1979). A quantity (0.25ml) of the homogenate and dilutions were pipetted on the surface of previously dried Baird-Parker agar plates and spread using a sterile bent glass rod. Duplicate plates were also prepared from each dilution. The plates were incubated for 24hrs at 37±0.5°C. Plates with 30-300 distinct colonies that were black and shiny with narrow white margins and surrounded by clear zones extending into the opaque medium were selected. The position of the colonies were marked and re-incubated for a further 24hrs at 37±0.5°C. All the colonies with the above characteristics that developed within the extended period of incubation were noted subjected to Gram's staining as well as coagulase and catalase tests for the confirmation of identity of the bacterium (Chesebrough, 2000).

Salmonella species

The food homogenate was prepared and transferred to a sterile 300ml-capacity sterile bottle. This was incubated at 37±0.5°C for 24hrs. A quantity (10ml) of the homogenate was transferred into a sterile bottle containing 100ml selenite-cystine broth and incubated at 42-43±0.5^{0}C for 48hr (FAO, 1979). On the other hand, DCA medium was inoculated with a loopful of the food homogenate and incubated for 18-24hrs at 37±0.5°C. The presence of characteristic colonies (black) of *Salmonella* on DCA was considered a positive result. For further confirmation, KIA plates were inoculated in duplicate with a suspected colony from DCA and incubated at 37±0.05°C for 18-24hrs. A positive result was indicated by a change in colour of the KIA medium (Chesebrough, 2000).

Results and Discussion

The results obtained from this study show that the sliced fruit samples examined were contaminated with bacteria, yeasts and molds (Tables 1-3). The presence of these micro-organisms could be as a result of unhygienic processing and handling, exposure during sales (hawking), cross contamination from other fruits and vegetables or improper storage conditions (Bryan *et al*., 1992; Taura *et al*., 2005; Kawo *et al*., 2006). The aerobic bacterial and fungal counts obtained in this study from the various sample sites were generally high (Tables 1-3). The values were generally above the limits recommended by FAO (1979), which states that the count should not exceed 10^4 and 10^2 cfu/g for bacteria and fungi respectively. The high bacterial, fungal and coliform counts observed in this study were generally lower than the findings of Richard and Thumberg (2004) who reported as high microbial counts as 10^8 - 10^9 cfu/g from sliced fruits collected from retail markets. In the present study, coconut had the highest bacterial count of 3.46×10^5 cfu/g (Table 2), followed by watermelon with 2.34×10^5 cfu/g (Table 2) while pineapple had the least of 1.70×10^5 cfu/g (Table 3). This could be attributed to the fact that most bacteria grow best at optimum temperature of 37^0C and pH values around 6.6-7.5 (FAO, 1979; Blomberg and Alder, 1992; Nester *et al*., 2001).

On the other hand, the fact that sliced pineapple had the highest fungal count of 2.88×10^5 cfu/g (Table 3) might be attributed to the low pH (4.29) recorded for the sample (Table 3). Coconut had the least fungal count of 1.27×10^5 cfu/g and pH of 6.60 (Table 1). This observation could be attributed to the fact that most fungi, particularly the yeasts and molds, grow best in acid environment of at a range of 4.5 to 6.6 under ambient condition (FAO, 1979; Blomberg and Alder, 1992; Nester *et al*., 2001; Kawo *et al*., 2006). The high frequency of isolation of *E. coli* (Tables 1-3) implicated the processing and rising water as possible sources of contamination of sliced fruits sold by vendors. Results from this study are similar to those of Blostein (1993) who reported *E. coli* and as being responsible for outbreaks involving pre-cuts fruits. The presence of *Staphylococcus* species might be explained by the fact that humans are the primary reservoirs of *S. aureus* in the nasal passage, hands and skins of healthy individuals (Nester *et al*., 2001; Abdulhadi *et al*., 2008).

As the fruits are being cut and handled with bare hands by the vendors, *S. aureus* could be introduced into the sliced fruits during slicing, processing or vending. The presence of *S. aureus* as shown in this study is similar to the work of Blostein (1993) who reported high frequency of *S. aureus* in food. *Staphylococcus aureus* are ubiquitous and are major food contaminants. They cause food spoilage and usually give rise to food-borne illnesses (Nester *et al*., 2001; Pelczar *et al*., 1986; 1993; Prescott *et al*., 2002). *Staphylococcus aureus* causes food intoxication, when allowed to incubate in certain foods and produces heat stable entero-toxins that render food dangerous even though it appears normal (Prescott *et al*., 2002).On the other hand, *Salmonella* species and *E. coli 0157:H7* were not isolated from any of the samples examined in the present study. This is in correlation with the findings of Oliveira *et al* (2006) who reported the presence of some food-borne bacteria with the exception of *Salmonella s*pecies from sugarcane juice in Brazil.

The detection of the species of *Aspergillus*, *Mucor* and yeasts in the samples might be associated with the water used in the processing and/or washing of the sliced fruits (Taura *et al*., 2005; Kawo *et al*., 2006). In addition, the environment also could have played a role in fungal contamination of samples. Poor sanitation may be an important factor in the contamination of unprocessed fruits. The method of collection and utensils used in the slicing together with inadequate storage and marketing may all contribute as sources of contamination. Therefore, use of clean water and utensils, adequate packaging and practice of good sanitary and hygiene within and around the environment could reduce recontamination of the ready-to-eat products. The ubiquity and the ability of *Aspergillus* and *Mucor* species to grow at low water activity make them important in post-harvest/processed contamination and decay (Blomberg and Alder, 1992; Pitt, 1995). *Aspergillus flavus* are well known producers of aflatoxins. Aflatoxins whose primary target is the liver are potent carcinogens, mutagens and teratogens and are acutely toxic to animals and human (Siame *et al*., 1996).

On the overall, the presence of these micro-organisms in this study could be associated with the general poor sanitary environmental conditions under which the fruits were handled (Muinde and Kuria. 2005).

Table 1: Physical, bacteriological and fungal characteristics of the fruit samples (Site A)

Sample	pH	Tem (^{O}C)	SPC (cfu/g)	FC (cfu/g)	Coliforms (MPN/g)	*E. coli*	*E. coli 0157:H7*	*Staph. Aureus*	*Sal.*	*Asp.*	*Mucor*	Yeasts
Pawpaw	6.14	32	2.01×10^5	1.89×10^5	1.233×10^3	+	ND	+	ND	+	ND	+
Watermelon	6.28	27	2.03×10^5	1.73×10^5	1.249×10^3	+	ND	+	ND	+	ND	+
Pineapple	4.59	32	1.81×10^5	2.24×10^5	1.001×10^3	+	ND	+	ND	ND	+	+
Coconut	6.60	28	2.95×10^5	1.27×10^5	1.667×10^3	+	ND	+	ND	ND	ND	+

Key: Tem = Temperature; SPC = Standard plate count; FC = Fungal count; *Sal.* = *Salmonella* species; *Asp.* = *Aspergillus* species; + = Detected; ND = Not detected.

Table 2: Physical, bacteriological and fungal characteristics of the fruit samples (Site B)

Sample	pH	Tem (^{O}C)	SPC (cfu/g)	FC (cfu/g)	Coliforms (MPN/g)	*E. coli*	*E. coli 0157:H7*	*Staph. Aureus*	*Sal.*	*Asp.*	*Mucor*	Yeasts
Pawpaw	6.09	31	2.15×10^5	2.20×10^5	1.238×10^3	+	ND	+	ND	+	ND	+
Watermelon	6.20	27	2.34×10^5	2.01×10^5	1.262×10^3	ND	ND	+	ND	+	ND	+
Pineapple	4.59	32	2.03×10^5	2.30×10^5	1.018×10^3	+	ND	+	ND	+	ND	+
Coconut	6.60	27	3.46×10^5	1.59×10^5	2.500×10^3	+	ND	+	ND	+	ND	+

Key: Tem = Temperature; SPC = Standard plate count; FC = Fungal count; *Sal.* = *Salmonella* species; *Asp.* = *Aspergillus* species; + = Detected; ND = Not detected.

Table 3: Physical, bacteriological and fungal characteristics of the fruit samples (Site C)

Sample	pH	Tem (^{O}C)	SPC (cfu/g)	FC (cfu/g)	Coliforms (MPN/g)	*E. coli*	*E. coli 0157:H7*	*Staph. Aureus*	*Sal.*	*Asp.*	*Mucor*	Yeasts
Pawpaw	6.04	27	1.89×10^5	2.60×10^5	2.25×10^3	+	ND	+	ND	ND	+	+
Watermelon	6.20	27	2.30×10^5	2.33×10^5	5.76×10^3	+	ND	+	ND	ND	ND	+
Pineapple	4.29	30	1.70×10^5	2.88×10^5	1.05×10^3	ND	ND	+	ND	+	+	+
Coconut	6.60	32	2.74×10^5	1.97×10^5	9.04×10^3	+	ND	+	ND	+	+	+

Key: Tem = Temperature; SPC = Standard plate count; FC = Fungal count; *Sal.* = *Salmonella* species; *Asp.* = *Aspergillus* species; + = Detected; ND = Not detected.

The microbial quality of fruits in their raw state, contaminated water or inadequate hand-washing by fruit vendors and the absence of individual sanitary practices are similar to the reports of Allwood *et al* (2004) who reported inadequate hand washing by fruit vendors as a vehicle for the transmission of contaminants. The organisms isolated may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes chronic or opportunistic infections. These highlight the need to safe guard the health of the consumers by employing good hygiene practice of these produce, which are consumed without heat treatment (Ofor *et al*., 2009). Results obtained from the elemental (Table 4) and proximate (Table 5) analysis showed that the sliced fruits are rich in essential mineral elements as well as carbohydrates (2.6-36.1%) and proteins (0.4-3.6), which indicate high nutritional quality, hence susceptibility to spoilage microorganisms. The presence of ash (1.0-2.8%) and crude fiber (0.5-6.3) in high quantity (Table 5) is important as they aid bowel peristalsis and provide minerals to the body respectively (Wardlaw and Kessel, 2002; Delvin, 2006). The moisture content (45.8-95.0) is high and is sufficient to encourage the growth and proliferation of food-spoilage and disease-causing bacteria and fungi (FAO, 1979).

Table 4: Mineral composition of the sliced fruit samples

Mineral (mg/l)	Pineapple	Pawpaw	Watermelon	Coconut
Calcium	3.19	1.00	1.49	1.00
Sodium	2.58	2.90	3.23	3.55
Potassium	12.41	10.69	9.66	6.21
Magnesium	18.25	5.38	4.50	7.25
Iron	0.22	0.28	0.22	0.33

Table 5: Proximate composition of sliced fruit samples

Component (%)	Pineapple	Pawpaw	Watermelon	Coconut
Moisture	88.5	91.8	95	45.8
Ash	1.5	2	1.0	2.8
Protein	1.22	0.6	0.4	3.6
Fats	0.9	0.5	0.3	5.4
Carbohydrates	6.28	4.6	2.6	36.1
Crude fiber	1.3	0.5	0.7	6.3

Conclusion and Recommendations

From the results obtained in this study, it can be seen that the sliced fruits obtained from the different locations in Kano metropolis are contaminated with microorganisms, some of which have adverse effects on the human health if consumed. This calls for improved surveillance system on fruits products and public health education as well as enlightenment of retailers and consumers. In addition, it is recommended that regular monitoring of the quality of sliced fruits for human consumption be introduced to improve adequate hygiene among vendors especially in the aspect of handling and cutting fruits with clean and sanitized utensils. Retailers and consumers should wash their hands and fresh fruits before preparation (such as slicing and peeling) and consumption; and discard sliced fruits left at room temperature for more than two hours to avoid any future pathogen outbreaks. Thus, it is recommended that the National Agency for Food and Drug Administration Control (NAFDAC) enforce good food hygiene practices to avoid contamination of sliced fruits.

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