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International Journal of Research in Biological Sciences

Original Article

Microbiological safety evaluation of street vended ready-to-eat fruits sold in Ota,

Ogun state, Nigeria.

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Received 03 July 2011; accepted 16 August 2011

Abstract

Microbiological safety evaluation of street vended ready to eat fruits was conducted in two vending sites, a local market and a University cafeteria. The mean total aerobic plate count ranges from $2.0x10^6$ to $8.2x10^8$ on Pineapple and Watermelon obtained from the local market and from $6.0x10^4$ to $2.7x10^7$ on apple and fruit salads from the University cafeteria. All the samples were contaminated with coliform and fungi with counts ranging from $2.2x10^5$ to $4.2x10^6$ and $2.0x10^1$ to $1.0x10^3$ in the samples from the cafeteria, and $2.0x10^5$ to $3.5x10^6$ and $2.0x10^2$ to $1.1x10^3$ for samples from the local market. Organisms identified include Bacillus spp 100%, *S. aureus* and Penicillium spp 80%, *Aspergillus niger* 60%, *E. coli*, Enterobacter, Salmonella, Klebsiella, Mucor spp 40%, *Pseudomonas aeruginosa*, Proteus, Micrococcus, and Lactobacillus spp 20%. The presence of coliform, and counts of $\geq 10^6$ in most of the samples is a reflection of the sanitary quality of the processing of the produce and calls for concern. Adequate training of food vendors to maintain high standard of personal and environmental hygiene, proper washing of fruits before consumption, regular washing of hands and effective application of hazard analysis critical control point (HACCP) will help control contamination of products.

Keywords: Ready to eat fruits, vendors, hygiene, coliform, contamination

1. Introduction

Ready-to-eat fruits are fruits that can be bought directly from street vendors or hawkers or at local markets and eaten immediately i.e. without necessarily having to cut, peel or rinse them before consumption as they have already been prepared by the vendors.

Fruits are an extraordinary dietary source of nutrients, micronutrients, vitamins and fiber for humans and are thus vital for health and well being. Well balanced diets, rich in fruits 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 [1]. Fruits 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 pathogens [2, 3, 4].

Over the last few years, there has been a significant increase in the consumption of sliced/ ready-to-eat fruits in Nigeria, because they are easily accessible, convenient, and most importantly, cheaper than whole fruits. Averagely in Nigeria, the smallest whole fruit- Apple costs more than a liter of fuel. To have a feel of a good meal taken along side some fruits costs up to One Thousand Naira (₦1000) about Six to Seven Dollars ($ 6-7) at least on fruits alone if one is to purchase whole fruit that is not cut to sizes. This is in a country with majority leaving on less than $1. Economic factors are therefore the major reasons that people have succumbed to consuming the already cut or sliced fruits.

The increased consumption, coupled with the associated risk of disease to which consumers may be exposed, is a matter of great concern. It is difficult for one to attest to the hygiene of the processors or to the sanitary conditions at points of

preparation. Moreover, the case is worsened by the fact that sliced fruit street vending is done without adequate storage conditions, thereby exposing the sliced fruits to flies and other disease-causing agents. The sliced/peeled fruits are processed and sold by unlicensed vendors with poor education levels and untrained in food hygiene [5, 6, 7]. The consumption of sliced/peeled fruits may thus potentially increase the risk of food-borne diseases caused by a wide variety of pathogens [8, 9].

There are different sources of microbial invasion of sliced produce. Pathogens may invade the interior surfaces of the produce during washing, peeling, slicing, trimming, packaging, handling and marketing [10, 7]. The use of dirty utensils, as well as the open display of street food produce encourages sporadic visits by flies, cockroaches, other insects, and dust [11]. Holding of sliced fruits that requires no further processing before consumption at ambient temperatures during retail maintains the produce at optimum temperatures for proliferation/invasion by pathogenic mesophiles [5]. Bacteria like Salmonella spp., Shigella spp., Campylobacter spp. and *Escherichia coli* can contaminate sliced fruits through contact with sewage and contaminated water [12, 13, 14, 15].

Fruits have been associated with outbreaks of food-borne disease in many countries. Organisms involved include bacteria, fungi, viruses and parasites [16, 17]. Kaplan and Campbell [18] implicated *Norovirus* in fruit salad. Outbreaks of salmonellosis have been associated with the consumption of cut watermelon in the United States of America [19]. This study was designed to assess the microbial contaminants of ready to eat fruits sold in Ota, Ogun State Nigeria, in order to highlight the health implications of consuming such unwholesome ready to eat fruits.

2. Materials and Methods

2.1 Source of samples

Samples were collected from two different locations, a local market in Ota and a University cafeteria. These two vending sites were chosen because the market is the major one in town and many vendors patronize the market for sales. The university services large population of Ota community.

2.2 Collection of Samples

A total of 60 samples comprising 12 each of five ready-to-eat/sliced fruits (apple, sliced watermelon, sliced pineapple, sliced pawpaw and packaged fruit salad) was purchased from vendors. The market was visited on three different occasions, during which 30 samples (10 per visit) was obtained from different vendors most of whom hawk round the market for sales. The university cafeteria was visited every two days for sample collection. All the samples were collected in polythene bags as sold and transported in a cold box to the laboratory for processing within 30 minutes-1hours after collection.

2.3 Isolation and Enumeration of Bacteria and Fungi

A sterile knife was used to cut 10g from each samples and then blended with sterile warring blender and homogenized in 100ml sterile peptone water. Each sample of Apple was rinsed out in 10ml sterile peptone water. The resultant homogenate was diluted 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. From the appropriate dilution, 0.1 ml was plated in duplicate onto the different media using the spread plate technique. Nutrient agar, Eosin Methylene blue agar and Potato Dextrose agar were inoculated for Total aerobic plate count, Coliform count and Fungal count respectively. Mannitol salt agar was used for isolation of Staphylococcus *aureus* while Salmonella Shigella agar was inoculated after 24hr pre-enrichment of sample homogenates in selenite-F broth, for isolation of Salmonellae. All inoculated plates were incubated at 37^{o}C for 24- 48 h to obtain viable bacterial counts, except, however, Potato Dextrose agar plates that was incubated at 28^{o}C for 72 h. Colonies were counted at the expiration of incubation period using the colony counter (Gallenkamp, England). Counts were expressed as colony forming unit per ml of sample homogenate (cfu/ml)

Characteristic discrete colonies on the different media were isolated and purified by repeated sub-culturing on nutrient agar. Pure cultures were stored on agar slants at 4^{0}C for further characterization.

2.3.1 Coliform test

The method as described by [20] was adopted. One (1) ml of each sample homogenate was transferred to sterile test tube containing Lactose broth and inverted Durham tubes. Incubation was for 24-48hrs at 37^{0}C before tubes were checked for gas production. This is the presumptive test. A loop full of inoculums from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plates and incubated at 37ºC and 44^{0}C for 24 hrs. Following incubation, colonies which formed bluish black colour with green metallic sheen, and reddish/ brown colonies were noted and isolated on agar slants (confirmatory test). Also colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 37ºC. The tubes were observed after 24hrs for gas production (completed test).

2.3.2 Identification of isolates

The bacteria isolates were identified based on standard methods of Speck [21]; Cowan, [22]. Isolates were Gram stained and specific biochemical tests performed to include Catalase activity, Sugar utilization, Oxidase test, Indole test, Urease test, Methyl red and Voges Proskauer tests Coagulase activity, Citrate utilization and Motility test.

Table1. Mean microbial load of ready-to-eat fruit samples

Sample	Local Market Mean count cfu/ml			University cafeteria Mean count cfu/ml		
	TAPC	TCC	TFC	TAPC	TCC	TFC
Apple	$7.0x10^{6}{}_{a}$	$3.4x10^{6}{}_{a}$	$1.1x10^{3}{}_{a}$	$6.0\ x10^{4}{}_{c}$	$2.2x10^{5}{}_{a}$	$1.0x10^{3}{}_{a}$
Fruit salad	$3.3x10^{8}{}_{b}$	$2.8x10^{6}{}_{a}$	$5.0x10^{2}{}_{a}$	$2.7x10^{7}{}_{d}$	$2.2x10^{6}{}_{a}$	$1.0x10^{2}{}_{a}$
Pawpaw	$2.1x10^{6}{}_{a}$	$2.0x10^{6}{}_{a}$	$5.0x10^{2}{}_{a}$	$4.8x10^{6}{}_{a}$	$4.2x10^{6}{}_{a}$	$2.0x10^{1}{}_{a}$
Pineapple	$2.0x10^{6}{}_{a}$	$2.0x10^{5}{}_{a}$	$2.0x10^{2}{}_{a}$	$2.0x10^{5}{}_{cf}$	$2.7x10^{6}{}_{a}$	$2.0x10^{2}{}_{a}$
Watermelon	$8.2x10^{8}{}_{b}$	$3.5x10^{6}{}_{a}$	$7.0x10^{2}{}_{a}$	$3.0x10^{6}{}_{a}$	$3.3x10^{6}{}_{a}$	$3.0x10^{2}{}_{a}$

TAPC= Total aerobic plate count TCC= Total coliform count TFC= Total fungal count a, b, c, d, f= Mean within column and row with the same letter for same count are not significantly different ($p>0.05$)

Table 2. Microbial isolates from ready-to-eat fruit samples.

Samples	Organisms isolated
Apple	*Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Bacillus Subtilis, Staphylococcus aureus, Bacillus spp,* Penicillium spp
Fruit salad	*Escherichia coli, Staphylococcus aureus, Micrococcus* spp, *Bacillus spp, Enterobacter, Aspergillus niger*
Pawpaw	*Salmonella* spp, *Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli,* Penicillium spp, Mucor spp
Pineapple	*Staphylococcus aureus, Bacillus subtilis,* Lactobacillus spp, Bacillus spp, Penicillium spp, Mucor spp, *Aspergillus niger*
Watermelon	*Salmonella* spp, *Bacillus subtilis*, *Staphylococcus aureus, Proteus spp, Enterobacter spp,* Penicillium spp, *Aspergillus niger*

Fungal isolates were identified based on their macroscopic and microscopic characteristics with reference to standard identification keys and atlas [23, 24].

2.3.3 Statistical analysis

The data obtained for counts were analysed by (ANOVA) analysis of variance [25]

3. Result

The mean microbial load of the fruit samples is as shown in Table1. It reveals that samples obtained from the local market had more microbial contaminants compared to samples from the University cafeteria, except however, for pawpaw. Table1 also reveal that all the samples from both vending sites had coliform and fungal contaminants. The mean total aerobic plate count of the samples range 10^{6} and above except for Apple and Pineapple from the University cafeteria. Watermelon and fruit salad had the highest level of contamination compared to other fruit samples.

A total of thirteen different organisms were isolated from the samples including Salmonella spp*, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, Bacillus spp, Proteus spp, Lactobacillus spp, Klebsiella, Enterobacter, Micrococcus spp*, Aspergillus niger,* Mucor and Penicillium spp (Table2). Bacillus spp occurred in 100% of the samples while *S.aureus*, Penicillium spp and *Aspergillus niger* contaminated 80% and 60% of the samples respectively.

4. Discussion

The consumption of sliced/peeled ready to eat fruits directly from street vendors or hawkers potentially increase the risk of food-borne diseases caused by a wide variety of pathogens, because it is difficult to attest to the hygiene of these vendors or to the sanitary conditions at points of processing as well as the packaging materials.

The significant difference observed in levels of contamination of products from the local market compared to the University cafeteria could be a reflection of the level of exposure and the handling processes in these two vending sites. In the market the products are opened as often as the customers' demand, open display of products to attract the

customers encourages sporadic visits by flies. The dusty, unhygienic market environments coupled with the poor handling by the vendors are factors contributing to the high microbial load. The common practice of using same bucket of water to wash all the fruits if it is ever washed at all [10] and the use of same utensils (knife) for cutting (cross contamination) [26, 29] could also be responsible for the microbial loads obtained even from products gotten from the University cafeteria. Vendors of ready to eat fruits usually make use of simple facilities like wheel barrows, trays, tables and make-shift stalls, thus further increasing the risk of food contamination. Pathogens may invade the interior surfaces of the produce during peeling, slicing, trimming and other processes like packaging, handling and marketing [26, 7].

The chances of contamination is heightened by the fact that sliced/peeled fruit street vending is done without adequate storage conditions, thereby exposing the sliced fruits to flies and maintains the produce at optimum temperatures for invasion and proliferation of contaminants, pathogenic mesophiles and other disease-causing agents. The sliced/peeled fruits are processed and sold by unlicensed vendors with poor education levels and untrained in food hygiene [11, 5, 6, 7]. Poorly processed street vended produce have been identified as an important cause of diseases in developing countries [9]. Fruits have been associated with outbreaks of food-borne disease in many countries; organisms involved include bacteria, fungi, viruses and parasites [16, 17, 27]. Ready to eat fruits (fruit salad) contaminated with *Norovirus* has been reported [18]. Outbreaks of salmonellosis have been associated with the consumption of cut watermelon in the United States of America [19, 28, 29].

Bacteria like Salmonella spp, Shigella spp., Campylobacter spp., and *Escherichia coli* can contaminate sliced fruits/ ready to eat foods through contact with sewage and contaminated water [12, 13, 14, 15].

Bacillus, penicillium and Aspergillus spp presented the highest percentage occurrence, being present in 100% to 60% of the samples. This could be due to the fact that these organisms are spore formers and are known common environmental contaminants; nevertheless, they have been implicated as food borne pathogens [30, 31, 32, 33, 34, 35, 36, 37]. *S. aureus* is a normal body flora and could have been introduced through unclean hands and mouth of the vendor and customers. The packaging materials are normally opened by squeezing/blowing air into it. *S. aureus* is an opportunistic pathogen and enterotoxigenic strains are known to cause serious food borne disease [38, 39]. Pseudomonas, *E. coli,* Klebsiella, Salmonella, Proteus and Enterobacter *spp* are environmental contaminants; they have been isolated from plants, human skin, animal and dairy products. Their presence in the ready to eat fruits could be through unclean hands of the vendor, contact with sewage and contaminated water [12, 13, 14, 15]. Cross-contamination of food during preparation has been identified as an important factor associated with food-borne illness [40, 29].

The presence of Salmonella spp, *E. coli,* Klebsiella and Enterobacter calls for concern as these organisms are frequently associated with poor sanitary practices and could be a pointer to danger of possible food borne infection. *E. coli* and Salmonella spp are especially of fecal origin and have been implicated in numerous food borne diseases [41, 39, 37].

The vendors, water and inadequate washing of hands and utensils appear to be the major hazard associated with these fruits and must be addressed properly. Vendors and consumers are advised to wash fresh fruits properly before peeling, slicing or cutting; fruits should be handled with clean and sanitized hands, utensils and surfaces and also stored refrigerated if any delay prior consumption. Good personal hygiene and effective hazard analysis and critical control point (HACCP) application reduces the chance of contamination of ready to eat fruits.

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Source of support: Nil; Conflict of interest: None declared