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Full-Length Research Paper

Bacteriological Assessment of Sliced Fruits (Pawpaw, Pineapple and Watermelon) Sold in Rumuokwuta Market

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ABSTRACT: The study was aimed at the bacteriological assessment of sliced fruits (pawpaw, pineapple and watermelon) sold at Rumuokwuta market using standard microbiological techniques. A total of eighteen (18) ready-to-eat fruits were screened for microbial counts. Ten gram (10g) each of the samples was cut with a sterile knife and was homogenized in 90 ml of distilled water in a beaker. Standard recommended procedures were used in the preparation of agars, serial dilution, inoculation and other biochemical analysis. The mean heterotrophic bacterial counts for pineapple, pawpaw and watermelon samples are 2.17 x10^8 cfu/g, 1.74 x10^8 cfu/g and 2.35 x10^8 cfu/g, respectively; the mean coliform counts pineapple, pawpaw and watermelon samples are 2.67 x10^8 cfu/g, 1.78 x10^8 cfu/g and 1.59 x10^8 cfu/g, respectively; the mean total *Salmonella-Shigella* counts for pineapple, pawpaw and watermelon are 3.58 x10^7 cfu/g, 3.00 x10^7 cfu/g and 5.33 x10^6 cfu/g, respectively. Most of the fruit samples from the vendors exceed the 0 -10^3 acceptable and 10^4 – 10^5 tolerable limit. The identified bacteria are *Escherichia coli*, *Bacillus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Salmonella* spp. and *Citrobacter* spp. This indicates that the fruits are contaminated and could cause health hazard if consumed. Based on the current findings, it is recommended that fresh and raw fruits should be properly washed before processing and consumption and Community Health Officers should educate vendors on the dangers of these organisms.

Keywords: Biochemical analysis, fruits, market

INTRODUCTION

Sliced fruits are fruits that have been cut open and also sliced into bits, but remain in its fresh state and displayed for sale and for consumption (Abadias *et al*., 2018). According to the International Fresh-Cut Produce Association (IFPA), fresh-cut fruit and vegetable products (FFVP) are defined as fruits or vegetables that have been trimmed, peeled or cut into a 100% usable product which has been packaged to offer consumers high nutrition and flavour, while still maintaining its freshness (Rico *et al*., 2007 and Pradas-Baena *et al*., 2015). Such fruits are directly purchased from the roadside vendors or hawkers or at local market without necessarily having to undergo any further treatment before consumption (Barro *et al*., 2016). Fruits are good dietary source of nutrients, micronutrients, vitamins and fiber for humans and are thus vital for health and are also rich in vitamins, minerals, antioxidants and other phytonutrients. Fruits are essential parts of people's diet and are vital for health and well-being as it reduce the risk of several diseases (Bukar *et al*., 2017).

In Nigeria, fruits are popularly displayed completely exposed for sales along busy and major streets and hawked by street food vendors in motor parks and markets. Well balanced diets, rich in fruits and

vegetables, are especially valuable for their ability to prevent vitamins C and A deficiencies and are also reported to reduce the risk of several diseases (Allamin *et al*., 2015). Sliced fruits are commonly processed and sold by unlicensed vendors with poor educational levels and untrained in food hygiene and have been on the increase in many developing countries due to lack of formal jobs for the working age groups (Bukar *et al*., 2016).

A great amount of income can be contributed to households by the sale of sliced fruits while providing a source of inexpensive nutritious meal. However, the increase in consumption of sliced fruits has been linked with a parallel increase in food borne illness (Eni *et al*., 2015). Fruit produce is known to carry a natural nonpathogenic micro flora, and have an epidermal layer of cells which provides a barrier for penetration of microorganisms. Cutting and slicing can eliminate the protections and microbes can invade the internal tissue. Thus, unsanitary processing and preservative methods could increase the possibilities of contamination (Allamin *et al*., 2015).

Poorly processed sliced fruits and open display of these sliced fruits have been identified to encourage sporadic visits by flies, cockroaches, rodents and dust and also an important cause of death in developing countries (Abadias *et al*., 2018). Most bacteria spp are known to be common environmental contaminants and bacteria that causes gastroenteritis can contaminate the sliced produce, thus exposing consumers to greater risk (Khali and Mazhar, 2019).

Bacteria which is a member of a large group of unicellular microorganisms that could be gram negative or gram positive and includes *Escherichia coli, Bacillus* spp*., Enterobacter*spp*., Pseudomonas aeruginosa, Proteus, Micrococcus, Lactobacillus* spp*., Staphylococcus aureus, Salmonella* spp., *Shigella* spp. and *Campylobacter* sp. etc., have been isolated and known to contaminate sliced fruits could be traced through contact with sewage, contaminated water, packaging nylon, tray and contaminated knifes (Abadias *et al*., 2018). Some of these bacteria are known to be spore formers and thus can easily contaminate sliced fruits when not properly processed. Sliced fruits are known to carry natural nonpathogenic micro flora; however, contamination with pathogens from humans or animals source can also occur sporadically during processing and packaging (Nwachukwu *et al.,* 2018).

Increase in the patronage of slice fruits have potentially increased human exposure to a variety of pathogens and have resulted in a potential increased risk of outbreak of illness associated with the consumption of these contaminated sliced fruits. These pathogens may invade the inner surface of the fruits during slicing or peeling. Studies carried out by Allamin *et al*., (2015) reveal the presence of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp., *Shigella* sp., *Listeria monocytogens* and *Salmonella* sp*.* as prevalent isolate bacteria in sold slice fruits.

The increase in the consumption of sliced fruits has been linked with increase in food-borne illnesses. Consumption of sliced fruit has been on the increase locally and globally. This is so because they are more convenient, easily accessible, and most especially cheaper than the whole fruits. Sliced fruits commonly sold in some markets which include pawpaw, pineapple and watermelon are processed and packaged by street vendors with poor education levels for food safety and handling hygiene (Buck *et al*., 2017).

The consumption of sliced pawpaw and watermelon may have potentially increase the risk of food-borne disease caused by variety of pathogens, cross contamination of fruits, unsanitary processing and use of dirty trays for display of fruits further increases the risk of contamination (Khali and Mazhar, 1994). Another major source is the water used in washing these slice fruits and the nylon used in packaging these fruits (Nwachukwu *et al*., 2018). Bacteria causing gastrointestinal infection can contaminate the sliced fruits and packaging nylon thus exposing the consumer to greater risk (Khali and Mazhar, 1994). It is difficult for one to attest to the hygiene of the processors or to the sanitary conditions at points of preparation. Moreover, this 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, which when consumed cause health risk (Beuchat, 2016); hence, a major reason for this study.

MATERIALS AND METHODS

Study area

The study area Rumuokwuta is located in Obio Akpor Local Government Area of Rivers State, and lies within latitude $4^0$50’26.178’ N and longitude 6^0 59’ 31.4268’ E.

Sample collection

A total of eighteen (18) samples of freshly processed sliced fruits (sliced pineapple, pawpaw and watermelon) was purchased from Rumuokwuta Market and then transported in a sterile bag to the Biology Laboratory in the Department of Science Laboratory Technology in Captain Elechi Amadi Polytechnic, Port Harcourt.

Sample preparation

Ten gram (10g) each of the sample was cut with a sterile knife and was homogenized in 90 ml distilled water in a

beaker. One millilitre (1ml) of the stock solution of the samples was pipette into 8 different test tubes containing 9ml of distill water, the resultant homogenate was diluted 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.

Microbiological analysis of fruit samples

Using a sterile 1.0ml pipette, 0.1ml of each of the dilution prepared was inoculated into eight (8) freshly prepared and surface dried nutrient agar plates, MacConkey Agar plates and Salmonella-Shigella Agar plates in duplicates for total heterotrophic bacterial count, total coliform count and Salmonella-Shigella counts analysis, respectively using spread plate method. The plates were then incubated at 37^{o}C for 24 hours.

Characterization and identification of organisms

Morphological features were observed and biochemical tests were conducted on the organisms to characterize and identify of the bacterial isolates from the sliced fruit samples. Morphological features of colonies observed were the size, surface texture, shape of colony, elevation of colonies, colour, and edge. The biochemical tests such as Gram staining, citrate utilization, indole production, methyl red test, Voges Proskauer test; Motility test, Triple sugar iron agar, catalase test, oxidase test, hydrolysis test, sugar fermentation test, crease test and coagulase test (slide method) were also conducted.

RESULTS

The results for total heterotrophic bacteria count, total coliform count and total *Salmonella-Shigella* counts for pineapple, pawpaw and watermelon samples are presented in (Table 1). The results of the total heterotrophic bacteria count shows that Pineapple 4 had the highest count of 2.35 x10^{8} cfu/g, while Pineapple 6 had the least count of 1.93 x10^{8}cfu/g with a mean count of 2.17 x10^{8} cfu/g; Pawpaw 3 had the highest bacteria count of 1.89 x10^{8} cfu/g, while Pawpaw 2 had the least count of 1.56 x10^{8}cfu/g with a mean count of 1.74 x10^{8} cfu/g; Watermelon 2 had the highest bacteria count of 2.88 x10^{8} cfu/g, while Watermelon 5 had the least count of 1.70 x10^{8}cfu/g with a mean count of 2.35 x10^{8} cfu/g.

The results of the total coliform count show that Pineapple 6 had the highest count of 2.80 x10^{8} cfu/g, while pineapple 1 had the least count of 2.43 x10^{8}cfu/g with a mean count of 2.67 x10^{8} cfu/g; Pawpaw 5 had the highest bacteria count of 2.48 x10^{8} cfu/g while Pawpaw 2 had the least count of 1.40 x10^{8}cfu/g with a mean count of 1.78 x10^{8} cfu/g; Watermelon 4 had the highest bacteria count of 2.34 x10^{8} cfu/g, while watermelon 5 had the least count of 9.25 x10^{7} cfu/g with a mean count of 1.59 x10^{8} cfu/g. The results of the total *Salmonella-Shigella* count shows that Pineapple 6 had the highest count of 1.00 x10^{8} cfu/g, while pineapple 1 had no count with a mean count of 3.58 x10^{7} cfu/g; Pawpaw 5 had the highest bacteria count of 3.85 x10^{7}cfu/g, while Pawpaw 3had the least count of 1.90 x10^{7} cfu/g with a mean count of 3.00 x10^{7}cfu/g; Watermelon 5 had the highest bacteria count of 1.60 x10^{7} cfu/g, while Watermelon 2 and 4recorded no count with a mean count of 5.33 x10^{6} cfu/g. The result of the characterization and identification of bacteria isolated from different street vended sliced fruits is presented in (Table 2). The identified bacteria are *E. coli*, *Bacillus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Salmonella* spp. and *Citrobacter* spp.

DISCUSSION

Microbes present in fruits in various studies directly reflects the sanitary quality of the cultivation water, harvesting, transportation, storage and processing of the produce (Beuchat, 1996; Ray and Bhunia, 2007). This study was undertaken to assess the bacteriological assessment of sliced fruits (Pawpaw, Pineapple and Watermelon) sold in Rumuokwuta Market in Port Harcourt, Rivers State.

In this study, the mean total heterotrophic bacterial count of the samples showed that pineapple, pawpaw and watermelon had mean counts of2.17 x 10^{8} cfu/g, 1.74 x 10^{8} cfu/g and 2.35 x 10^{8} cfu/g, respectively. The counts obtained in this study were higher than the 8.2 x 10^{5} cfu/g for pineapple and watermelon and 6.9 x 10^{5} cfu/g for pawpaw as reported by Ugwu and Edeh, (2019). These values from different markets are high and could be as a result of mishandling and the practice of using the same bucket of water to wash all the fruits (Khali and Mazhar, 1994) as well as cross contamination using the same utensils to cut and display the fruits (Ugwu and Edeh, 2019).

In this study, the mean total coliform count of the samples showed that pineapple, pawpaw and watermelon had mean counts of 2.67 x 10^{8}cfu/g, 1.78 x 10^{8}cfu/g and 1.59 x 10^{8}cfu/g, respectively. Mahfuza *et al*., (2016) had reported that the washing and processing water contaminated with fecal coliform is one of the major sources for presence of coliforms in street foods.

In this study, the mean total *Salmonella-Shigella* count of the samples showed that pineapple, pawpaw and watermelon had mean counts of 3.58x10^{7} cfu/g, 3.00x10^{7} cfu/g and 5.33 x10^{6} cfu/g, respectively. Mahfuza *et al*., (2016) had reported a total *Salmonella-Shigella* count of 7.1x10^{1} cfu/g in pineapple. The presence of *Salmonella* and *Shigella* into fresh cut fruits and vegetables were due to contamination of tools and knives (Barro *et al*., 2006).

Table 1: Mean bacterial counts obtained from different street vended fruits.

Sample Vendor		THBC cfu/g	Mean cfu/g	TCC cfu/g	Mean cfu/g	TSS cfu/g	Mean cfu/g
Pineapple	1	$2.23x10^8$	$2.17x10^8$	$2.43 x10^8$	$2.67x10^8$	-	$3.58x10^7$
Pineapple	4	$2.35 x10^8$		$2.79 x10^8$		$7.50 x10^6$	
Pineapple	6	$1.93 x10^8$		$2.80 x10^8$		$1.00 x10^8$	
Pawpaw 2		$1.56 x10^8$	$1.74x10^8$	$1.40 x10^8$	$1.78x10^8$	$3.25x10^7$	$3.00x10^7$
Pawpaw 3		$1.89 x10^8$		$1.47 x10^8$		$1.90 x10^7$	
Pawpaw 5		$1.78 x10^8$		$2.48 x10^8$		$3.85 x10^7$	
Watermelon	2	$2.88 x10^8$	$2.35x10^8$	$1.51 x10^8$	$1.59x10^8$	-	$5.33x10^6$
Watermelon	4	$2.48 x10^8$		$2.34 x10^8$		-	
Watermelon	5	$1.70 x10^8$		$9.25 x10^7$		$1.60 x10^7$	

THBC: Total Heterotrophic Bacteria Count; TCC; Total Coliform Count; TSSC: Total; *Salmonella-Shigella* Count

Table 2: Characterization and identification of bacteria isolated from different street vended sliced fruits.

Code	Size	Colour	Shape	Elevation	Surface	Edge	Texture	Gram Reaction	Catalase	Coagulase	Indole	Citrate	Methyl red	Voges p	TSIA			Gas Production	Probable organism
															Slant	Butt	H_2S		
Pineapple 6 SSA	3mm		Circular	Raise	Smooth	Entire	Wet	-rods	+	-	-	-	+	-	R	Y	+	-	*Salmonella* sp.
Pineapple 1 MAC	3mm	Pink	Circular	Raise	Rough	Entire	Wet	-rods	+	-	+	-	+	-	R	R	-	-	*Escherichia coli*
Pineapple 4 MAC	5mm	Pink	Circular	Flat	Rough	Entire	Wet	-rods	+	-	-	+	+	+	R	R	-	-	*Enterobacter* sp.
Pineapple 6 SSA	5mm	Pink	Circular	Raise	Smooth	Entire	Dry	-rods	+	-	+	-	-	+	R	R	-	-	*Klebsiella* sp.
Pineapple 1 SSA	5mm	Pink	Circular	Raise	Smooth	Entire	Dry	-rods	+	-	+	+	+	+	R	Y	-	-	*Klebsiella* sp.
Watermelon 5NA	4mm	Creamy	Circular	Raise	Rough	Entire	Wet	-rods	+	-	-	+	+	+	R	R	-	-	*Pseudomonas* sp.
Paw-paw 1 NA	5mm	Creamy	Circular	Flat	Smooth	Entire	Dry	-rods	+	-	+	+	+	+	R	Y	-	-	*Bacillus* sp.
Paw-paw 2NA	5mm	Creamy	Circular	Raise	Smooth	Entire	Dry	-rods	+	-	+	+	+	+	R	R	-	-	*Citrobacter* sp.
Paw-paw 3NA	8mm	Creamy	Circular	Flat	Smooth	Entire	Dry	-rods	+	-	+	+	+	+	R	R	-	-	*Citrobacter* sp.
Pineapple 1 NA	6mm	Creamy	Circular	Flat	Smooth	Entire	Dry	+rods	+	-	-	+	+	+		Y	-	-	*Bacillus* sp.
Pineapple 4 NA	6mm	Creamy	Circular	Flat	Rough	Entire	Dry	+rods	+	-	+	+	+	+	Y	Y	+	-	*Bacillus* sp.

Key: NA=Nutrient Agar; MAC MacConkey Agar; SSA *Salmonella–Shigella* Agar; +Positive; -Negative; R =Red; Y=Yellow; TSIA=Triple Sugar Iron Agar.

In addition, flies sometimes attack knives used for cutting and chopping of fruits and vegetables (Mensah et al., 2002). Beuchat (1995) and Gayler *et al*., (1995) also stated that *Salmonella* spp. and *Shigella* spp. can infect fresh cut fruits through contact with contaminated water. Generally, these counts from the different vendors and exceed the International Commission on Microbiological Specification for Food (ICMSF, 1996) limit that ready-to-eat foods with plate counts between 0 - 10^3 is acceptable, within 10^4 –10^5 is tolerable and 10^8 and above is unacceptable. From the results obtained, the level of contamination is unacceptable based on the recommended standards.

The study also revealed the presence of *Escherichia coli*, *Bacillus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Salmonella* spp. and *Citrobacter* spp. from the pineapple, pawpaw and watermelon samples. This is in agreement with the work done by Ikpebivie *et al*., (2020) and Olu-Taiwo *et al*., (2021). According to Daniyan and Ajibo (2011), the presence of these organisms in the fruits could have been through faecally-polluted water used in washing utensils such as knives, trays and polyethene bags used for the packaging of the fruits after slicing or cutting and also exposure to low temperatures which encourage the growth of these pathogens.

The dusty environment of the motor parks, busy roads coupled with unclean water used to sprinkle on the fruits could be part of the contributing factors that aid the survival and multiplication of these microorganisms (Oranusi and Braide, 2012). The nutritional composition and availability of water in the fruit which are essential for the growth and survival of the microorganisms could have also influence the presence the organisms (Nwachukwu and Osuocha, 2014). The implication is that the contaminated fruits samples could readily serve as a vehicle to transmit these pathogens to the consumers (Orji *et al*., 2016). Again, all these microorganism causes food borne infection or intoxication and diarrhoeal diseases (Mahfuza *et al*., 2016). During this study, it is important to note that at the time of sampling, these fruits did not show any sign of spoilage; hence, the outward appearance cannot be used to determine the good quality of any fruits, and therefore should be thoroughly washed before it is consumed.

Conclusion

From the results obtained above, it clearly shows that these fruits are contaminated and may cause health hazard when consumed. Therefore, good processing and proper hygienic handling methods should be used in sliced fruits preparation to avoid bacterial contamination. Health Officers should help out in the monitoring these vendors in order to minimize the risk of disease outbreak associated with consumption of contaminated fruits.

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