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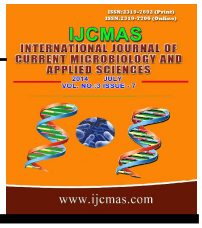

Original Research Article

Evaluation of Microbial Contamination of Street-Vended Fruit Salad in Calabar, Nigeria

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A B S T R A C T

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 using microbial limit test. A statistically convenient sample size (N=20) of pre-packaged fruit salad made from different combinations of fresh fruits were evaluated for microbial contaminants using standard plate techniques. 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 high microbial load in 90% of the samples evaluated. Heterotrophic bacteria contaminated 90% of the samples, non-fecal coliform (80%), fecal coliform (60%), molds (70%) and yeasts, (50%). Total and fecal coliform counts ranged from 3.7 x 10^5 cfu/g to 6.8 x 10^5 cfu/g and from 3.2 x 10^5cfu/g to 5.8 x10^5cfu/g respectively, while total staphylococcal and fungal counts ranged from 3.4cfu/g to 6.5cfu/g in both cases. 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.

Keywords

Fruit salad, contaminant, microbiological safety, coliform,

Introduction

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, fruit salad is classified as ready-to-eat street food because they are often bought directly from street vendors or hawkers or at road side kiosks and eaten immediately in the form they are bought without further processing like washing, peeling or slicing.

Many consumers patronize the street vended fruit salad due partly to the fact that they are cheaper than whole fruits, convenient to eat and partly because they are easily available. Fruit salad is usually a combination of various fresh fruits such as apples, water melon, pineapples, cucumber, pawpaw and orange. They are usually sliced into small

pieces and packaged in small transparent plastic bowls. The salad can be eaten using fork or tooth pick, with or without milk added to it.

Since fruit salad consists of different fruits, the overall nutritional status of the salad reflects the nutritional quality of the individual fruits used in its preparation. According Edward *et al.* (2012), fruit salad is low in cholesterol, sodium and saturated fat but high in vitamins A, C and D, manganese, copper and dietary fiber.

In Calabar, fruit salad is prepared and sold in road side kiosks, recreational areas and busy streets around the University of Calabar. The different fruits used in preparing the salad are usually kept on the ground near the slicing tables without any form of protection. Hence the microbiological quality of the prepared fruit salad remains doubtful.

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 fruit salad in Calabar, Nigeria, from September to December, 2013. The decision to carry out the research within this period is due to the fact that this last quarter of the year is the festive period in Calabar with upsurge of people from different parts of the world coming for carnival and street party. Consequently, there is always increase in the demand and consumption of street vended mixed fruits during this period. Therefore this work is of utmost importance during and after this period, and the findings will enable people to appreciate the possible health implications of eating microbiologically unsafe fruit salad.

Materials and Methods

Sample collection

A total of 20 pre-packaged fruit salad samples comprising cucumber, pawpaw, pineapple and water melon were obtained randomly from three locations popularly known for vending fruit salad. All the samples were collected wholly in small transparent plastic bowls as sold and transported in ice-packed cooler to the laboratory where they were analyzed within 1 hour after collection.

Microbiological analysis of the collected samples

Microbiological analysis included isolation, enumeration and identification of pathogens using standard procedures. Media used for

this analysis were the Oxoid brand of nutrient agar, peptone water, eosin methylene blue agar, cetrimide agar, mannitol salt agar and Sabouraud dextrose agar. All the media were prepared according to manufacturers' instruction. For the isolation and enumeration of pathogens in the samples, each fruit salad sample was blended in a sterile blender and 1g of the homogenate 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 media using plate technique. Bacterial plates were incubated at 37°C for 24-48h, while the fungal plates were incubated at 28°C for up to 5 days. After incubation both the bacterial and fungal plates were examined for the presence of discrete colonies. Colonies were counted using the colony counter (Galenkamp, England) and expressed as colony forming unit per gram (cfu/g) of sample homogenate.

Specifically, total aerobic counts were performed on nutrient agar, while *E.* coil and fungi were enumerated on eosin methylene blue agar and Sabouraud dextrose agar respectively. Mannitol salt agar and MacConkey agar were used to enumerate *Staphylococus 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.

Identification of isolates

The methods of Oranusi *et al.,* (2004) was employed for the identification of coliforms and aerobic baccteria while the funqal isolates were identified based on the taxonomic schemes described by Fawole and Oso (1988). The identities of coliforms and aerobic bacteria were confirmed using the identification aid outlined in Bergey's Manual for Determinative Bacteriology (Holt *et al.,* 1994).

Results and Discussion

The present investigation reveals high microbial load in the fruit salad studied. The microbial contaminants consist of comprising different pathogens such as *Escherichia coli, Staphylococcus aureus, Streptococcus* sp., *Bacillus cereus, Salmonella* sp., *Pseudomonas* sp., molds and yeasts. The mean microbial counts of the fruit salad samples is presented in Table 1.

The result shows that 90% of the samples had high total viable counts ranging from 3.49×10^5 to 6.8×10^5 colony forming units per gram of the salad homogenate. The presence of these organisms in high numbers in fruit salad is of serious safety concern about the consumption of street vended foods. Some of the organisms encountered in this study are similar to those reported in similar studies from different countries (Mahale, 2008, Edward *et al*., 2012; Rashed, 2013; Nicholas, 2000). This confirms that fruit salad is highly susceptible to microbial contamination. Microorganisms can contaminate fruit salad through various sources such as unsanitary conditions, unhygienic handling and processing, use of poor quality source of water to wash the fruits, cross contamination from other fruits and vegetables or the use of dirty processing utensils like knives, cutting sticks, slicing tables and trays. In this study, *Bacillus cereus, Salmonella* sp., *and Escherichia coli* were isolated from the fruit salad. These

organisms are known causes of food borne illnesses and might have been introduced into the food by unhygienic processing, hence, consumers of fruit salad are exposed to the risk of food borne illness.

The presence of fecal coliform *E. coli* in the salad samples is an indication of fecal contamination often associated with fecally contaminated water, waste water or sewage sludge. It is possible that vendors did not wash the fruits properly or they might have used fecally contaminated water or waste water to wash the fruits used in preparing the salad. Improper washing of the component fruits adds these microorganisms into the salad resulting in contamination. The presence of coliform in fruit salad is not allowed by safe food consumption standard (Andres *et al*., 2004), Similarly Gulf standard (2000) states that the maximum permissible fecal coliform load in fruit juice is zero (Table 2). In this study however, the total fecal coliform counts ranged from 1.8 $x10^5$ to 2.6 x 10^5 cfu/g of the samples which is far above the limit recommended for fruit related food. This implies that most of the fruit salad sold in Calabar are unsuitable for consumption.

Table 3 shows the extent of microbial contamination of the fruit salad studied. Out of the 20 samples analyzed, 18 samples (90%) were contaminated with various kinds or microorganisms. From the contaminated samples, *E. coli* comprised 40% while 55% and 35% of the samples yielded *Staphylococcus aureus* and *Bacillus cereus* respectively. Contamination with *S. aureus* might be through handlers while B. *cereus* might have come in contact with the fruit -through the soil. The values of microbial contaminants obtained in this study are less than those reported by Edward *et al*. (2012) in a similar study in Port Harcourtt, Nigeria. The differences might be due to disparity in the processing methods and the sanitation of the production area and personal hygiene of the vendors.

It is commonly observed that fruits used-in preparing the salad are displayed on the ground by vendors for a long time. Such prolonged exposure of the fruits might permit entry of microorganisms and their subsequent internalization in the fruit tissues. 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.

Table.1 Mean viable counts ($x10^5$cfu/g) of microorganism in fruit salad

Sample code	Total count	Coliform count	Fecal coliform	Total Staphylococcal count	Total fungal count
1	3.2 ± 0.03	1.8 ± 0.11	1.3 ± 0.04	0.2 ± 0.07	1.5 ± 0.68
2	2.0 ± 1.16	1.4 ± 0.76	2.6 ± 1.13	1.7 ± 0.38	2.3 ± 0.10
3	6.5 ± 0.72	2.0 ± 0.14	0	0	1.9 ± 0.46
4	4.7 ± 1.13	1.3 ± 0.56	2.0 ± 0.63	2.4 ± 0.42	0
5	5.6 ± 0.36	3.3 ± 0.08	1.4 ± 0.18	0	2.7± .35
6	2.3 ± 0.14	2.7 ± 0.17	2.3 ± 0.53	3.4 ± 0.68	2.7 ± 1.67
7	1.7 ± 0.03	0.7 ± 0.43	3.1± 0.83	2.8 ± 0.16	1.6 ± 0.03
8	3.1 ± 0.19	2.0 ± 0.02	1.6 ± 0.47	0	2.8 ±0.88
9	6.0 ± 2.43	2.8 ± 4.36	2.5 ± 3.10	1.0 ± 0.63	3.4± 1.17
10	4.2 ± 0.05	1.7 ± 0.11	1.3 ±0.72	0	2.0± 2.48
11	1.6 ± 1.71	1.0 ± 0.58	1.8±1.23	0.8 ± 2.70	1.1± 0.08
12	3.7 ± 0.06	2.6 ± 0.17	3.2± 1.48	0.6± 5.25	2.7± 0.44
13	2.5 ± 0.13	2.0± 1.86	1.2± 5.01	2.5± 0.76	1.4± 1.72
14	0	0	0	0	0
15	2.8 ± 0.24	2.2 ± 0.01	1.9 ± 0.04	1.3 ± 0.25	21. ± 0.17
16	2.4 ± 0.08	0	0	0	0
17	0	0	2.3 ± 0.72	2.6 ± 0.03	1.8 ± 0.17
18	4.1 ± 0.04	3.0 ± 0.11	1.7 ± 0.02	2.3 ± 0.48	2.8 ± 0.33
19	5.3 ± 0.45	2.7 ± 0.37	2.2 ± 0.73	1.6 ± 0.08	2.1 ± 0.68
20	3.1 ± 0.18	2.3 ± 0.05	1.8 ± 0.06	2.0 ± 0.13	1.5 ± 0.02

Table.2 Recommended microbiological standards for any fruit juice

Parameter	Total viable Count	Non-fecal Coliform	Fecal coliform	Staphylococci
Maximum bacterial load anticipated	$5.0x10^7$	10	0	100
Maximum bacterial load permitted	1.0×10^4	100	0	1.0×10^3

* Numbers are per ml of juice Source: Gulf standards (2000).

Table.3 Occurrence of microbial contaminants in the fruit salad studied

Total No. of samples studied	20
Total No. of samples contaminated	18
Percentage of the sample contaminated	90
Number and percentage of samples contaminated with:	
Bacillus cereus	10(50.0)
Staphylococcus aureus	7 (35.0)
Escherichia coli	11(55.0)
Pseudomonas sp.	8(40.0)
Aspergillus niger	5(25.0)
Rhizopus sp	6(30.0)
Enterobacter sp.	5(25.0)
Streptococcus sp.	7(35.0)

* Some samples yielded more than one microbial type.

Table.4 Extent of contamination of fruit salad by different microbial groups

Number of samples analyzed	20
Number of samples contaminated	18
Number & percentage of samples contaminated with:	
Heterotrophic bacteria	18(90.0)
Non-fecal coliforms	16(80.0)
Fecal coliforms	12(60.0)
Molds	14(70.0)
Yeast	10(50.0)

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