Journal of Applied Life Sciences International

23(5): 1-7, 2020; Article no.JALSI.57158
ISSN: 2394-1103

Bacterial Assessment of Unpasteurized Fruit Juices Sold in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author CPA designed the study, managed the literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VNA and KN managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/JALSI/2020/v23i530159
Editor(s):
(1) Dr. Vasil Simeonov, University of Sofia "St. Kliment Okhridski", Bulgaria.
Reviewers:
(1) O. Okpo, Ngozi, Federal College of Agricultural Produce Technology, Nigeria.
(2) Krishnan Mahalakshmi, Sree Balaji Dental College and Hospital, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57158

Original Research Article

Received 26 March 2020
Accepted 01 June 2020
Published 12 June 2020

ABSTRACT

This study assessed the bacterial quality of fresh fruit juices sold in some restaurants in Port Harcourt, Nigeria. A total of twenty (20) fruit juice samples were collected from four (4) different types of fruit juices sold at four (4) different restaurants. The fruits that were used for this study are: Orange, Watermelon, Pineapple and Tiger nuts. Samples of the fruit juices were collected for bacteriological assessment using heterotrophic plate count and most probable number techniques, while CHRO Magar for *E. coli* and other coliforms was used for the detection of *E. coli*. The results showed that *E. coli*, *Bacillus* spp, *Klebsiella spp*, *Streptococcus* spp and *Staphylococcus* spp were present in the fruit juices. The results of the heterotrophic plate count showed that the bacterial count ranged from 2.7 x 10^2 (Orange) to 7.1 x 10^3 (Tiger nuts), while the results of the total coliform count ranged from 11 MPN/100 ml to 28 MPN/100 ml. However, the study revealed that the total *E. coli* count ranged from 0 to 7. The number of *E. coli* in each of the fruit juices and restaurants ranged from 6 to 13, *Bacillus* spp 12 to 19, *Klebsiella* spp 4 to 10, *Streptococcus* spp 6 to 11 and *Staphylococcus* spp 4 to 13. *Bacillus* spp had the highest percentage (31.4%), followed by *Staphylococcus* spp (20.1%). The highest percentage of bacteria was recovered from Tiger nuts (29.4%), followed by Watermelon (28.4%). Regular monitoring of the quality of fresh fruit juices sold in restaurants in Port Harcourt and other parts of Nigeria should therefore be enforced.

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Keywords: Fruit juices; E. coli; contamination; bacteria; hygiene; unpasteurised; restaurants; coliform.

1.INTRODUCTION

Fruit juices are nutritious for human beings, however during the preparation, packaging and storage processes, the hygiene practised could make them prone to microbial contamination. Fruits are exposed to contamination by microorganisms through contact with water, dust, soil, handling (at harvest) and during postharvest processing. As a result, they harbour different microorganisms, including human and plant pathogens [1,2,3]. In Port Harcourt, various kinds of fruit juices sold by different restaurants are consumed by the residents of the city. These fruit juices include Orange, Watermelon, Tiger nut and Pineapple [4].

Objects, such as dishes, knives and other fomites could contaminate the fruits and make them unsafe for consumption. These fomites may also have a role in the spread of some pathogenic bacteria, such as *Vibrio, Escherichia coli (E. coli), Salmonella and Shigella,* which could cause diseases in humans, as well as cause fruit spoilage [5]. Prior to pasteurization, it has been recorded that fruit juices contain a microbial load, which represents microorganisms that are normally found on fruits during harvest; inclusive are contaminants added after harvest. Pasteurization will reduce the microbial load significantly and lengthen the shelf-life of the product. Most of the cases of growth of bacteria in fruit juices have been reported and some of them describe human illness due to unpasteurized juices [6,7].

In many developing countries, the manufacturers of fruit juices are not very concerned about the microbiological safety and hygiene of fruit juices. This is because of lack of enforcement of the relevant laws in those countries, which Nigeria is one of them. However, in developed countries, the quality of fruit juices is strictly maintained as those countries are under several laws and regulations [8,9]. In a study carried out on orange juice prepared and sold in Port Harcourt, Nigeria, the researchers reported the presence of bacteria, such as *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Enterococcus* spp. and *E. coli*. Apart from bacteria, they recovered some fungal isolates, such as *Aspergillus* spp., *Penicillium* spp., *Saccharomyces* cerevisae and *Trichoderma* spp. From the industrially processed juice, they reported that *Saccharomyces cerevisae* was the highest isolated fungus, while from the locally made fruit juice, the highest fungi isolated were *Aspergillus* spp. and *Penicillium* spp. [10]. Also, another group of researchers in Port Harcourt, Nigeria, who worked on unpasteurised fruit juices, such as orange, watermelon and pineapple isolated bacteria, which are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella enteric*a and *Shigella* spp. The fungi they isolated were identified as *Penicillium* sp., *Fusarium* sp. and *Aspergillu*s sp. They stated that the high microbial load and presence of enteric pathogens indicate poor sanitary quality of the products [4].

In Port Harcourt, Nigeria, most of the fruit juices vended in restaurants are not pasteurised and this business is on the increase because of high demands by consumers as fruit juices are nutritious. As a result, this study was carried out in order to assess bacterial quality of unpasteurised fruit juices produced and vended in restaurants in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The present study was carried out in Port Harcourt, Rivers State, Nigeria. Port Harcourt is the Capital of Rivers State, which is one of the States in Nigeria. In Port Harcourt restaurants, fruit juices are processed and sold, some of which are unpasteurized.

A total of twenty (20) fruit juice samples were collected from four (4) different types of fruit juices sold at four (4) different restaurants in Port Harcourt, Nigeria. The following fruit juices were used for this study: Orange, Watermelon, Pineapple and Tiger nuts. In this study, the names of the four restaurants are not mentioned because of security purposes. Hence, the names of the restaurants are labelled as A, B, C and D. Twenty-five millilitres (25 ml) of each of the fruit juices were collected into sterile containers and transported to the laboratory in a cooler containing ice packs. The samples were analysed in Medical Microbiology Laboratory in the Department of Medical Laboratory Science, Rivers State University within 3 hours after collection.

2.2 Sample Analysis for the Cultivation and Detection of Microorganisms

Before the samples were analysed, all media and reagents were prepared in the laboratory according to manufacturers' instructions and directions. For instance, media such as Nutrient agar (used for heterotrophic plate count), MacConkey agar, MacConkey broth and CHRO Magar for *Escherichia coli* and other coliforms (CHRO Magar ECC) (used for most probable number technigue) were prepared and used following manufacturers' instructions and directions. After the preparation of the media, the media plates (petri dishes) were labelled clearly and stored appropriately in the refrigerator at 4-6°C [11,12].

Data were acquired from heterotrophic plate count (HPC), total coliform count (TCC) and Total *E. coli* Count (TEC); the TCC and TEC were carried out using the most probable number (MPN) technique, which was statistically determined by the use of MacCrady table. For HPC technique, appropriate serial dilutions of all the samples were carried out and the selected dilution was plated using the pour plate method. Then, enumeration of the total viable count was done using the Nutrient agar. All cultures were incubated at 37°C for 18 to 24 hours [12].

3. RESULTS

3.1 Bacteria Isolated from the Various Restaurants and Fruit Juices

The bacteria that was most isolated is *Bacillus* spp., followed by *Staphylococcus* spp. The number of the bacteria isolated ranged from 2 to 6 and 1 to 4; *Bacillus* spp. and *Staphylococcus* spp., respectively. Fruit juices from all the Restaurants harboured bacteria. Among the fruit juices, Tiger nut had the highest number of bacteria, followed by Watermelon (Table 1).

3.2 Percentage of Bacteria Isolated from Each Fruit Juice

Fig. 1 shows the percentages of bacteria isolated from each of the fruit juices. The percentages of bacteria ranged from 15.7% to 29.4%. Tiger nut juice had the highest percentage (29.4%), followed by Watermelon (15.7%).

3.3 Percentage of the Individual Bacteria Isolated from All the Fruit Juices

Fig. 2 shows the individual bacteria isolated from all the fruit juices. According to the results, *Bacillus* spp. had the highest percentage (31.4%), followed by *Staphylococcus* spp. (20.1%).

3.4 Enumeration of Bacteria Using the Heterotrophic Plate Count (HPC) Technique

According to Table 3, the HPC ranged from 2.7×10^2 to 7.1×10^3. Restaurant C had the highest HPC (7.1×10^3) from Tiger nut, while restaurant B had the lowest HPC (2.7×10^2) from Orange juice.

3.5 Enumeration of Bacteria Using the Most Probable Number (MPN) Techniques

Table 4 shows the Enumeration of bacteria using the MPN technique. According to the results, Restaurants A and B had the highest total coliform counts (28) from Tiger nut and Watermelon, respectively. The total *E. coli* count showed that *E. coli* was more in fruit juices isolated from Restaurant D.

Table 1. Bacteria isolated from the various restaurants and fruit juices

Bacteria	Restaurants (Locations)															
	A				B				C				D			
	O	WM	P	TN	O	WM	P	TN	O	WM	P	TN	O	WM	P	TN
Escherichia coli (*E. coli*)	2	3	2	3	1	2	2	3	1	4	2	3	2	4	2	3
Bacillus spp	3	4	4	6	4	5	4	5	3	3	5	4	2	6	2	4
Klebsiella spp	1	2	2	2	1	1	-	-	2	3	4	2	-	4	2	1
Streptococcus spp	2	1	3	3	1	2	2	3	2	1	2	3	1	2	3	2
Staphylococcus spp	2	3	3	4	1	4	4	3	-	2	3	2	1	2	3	4

Keys: O – Orange; WM – Watermelon; P – Pineapple; TN – Tiger nut. A, B, C, D represent the Restaurants

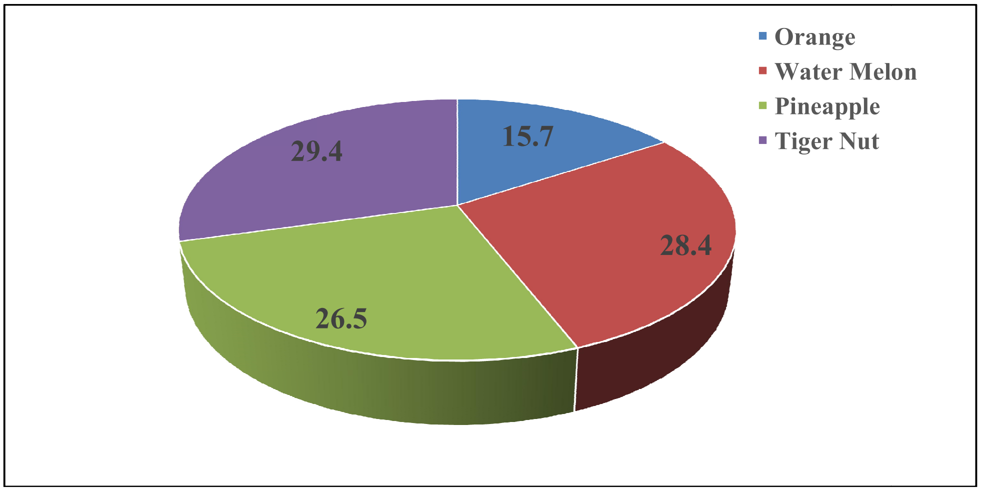

Fig. 1. Percentage of bacteria isolated from each fruit juice

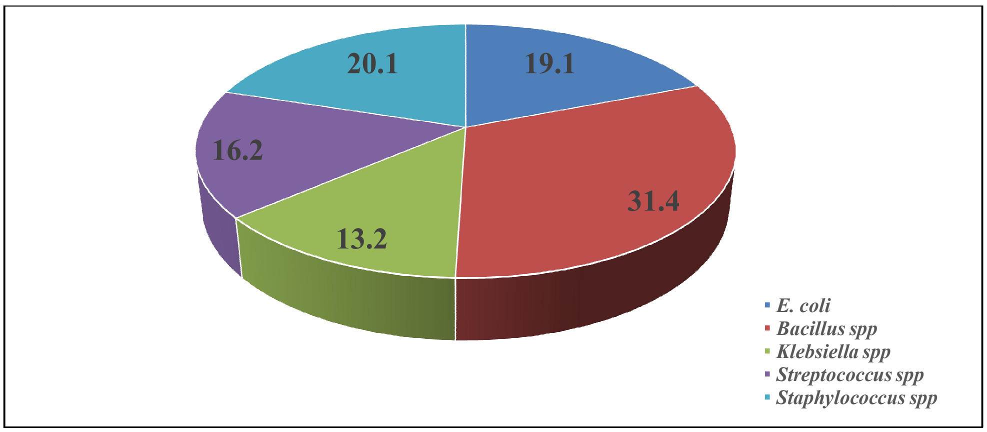

Fig. 2. Percentage of the individual bacteria Isolated from all the fruit JUICES

Table 2. Enumeration of bacteria using the heterotrophic plate count (hpc) technique

Fruit Juices	HPC			
	A	B	C	D
Orange	2.9×10^2	2.7×10^2	3.1×10^2	3.2×10^2
Watermelon	3.1×10^3	3.0×10^3	3.8×10^3	3.7×10^3
Pineapple	2.6×10^3	2.6×10^3	5.4×10^3	2.0×10^3
Tiger Nut	3.5×10^2	4.1×10^3	7.1×10^3	5.0×10^3

A, B, C, D represent the Restaurants

Table 3. Enumeration of bacteria using the most probable number (mpn) techniques

Fruit Juices	MPN/100 ml							
	Total Coliform Count (TCC)				Total *E. coli* Count (TEC)			
	A	B	C	D	A	B	C	D
Orange	11	11	14	17	3	0	0	7
Watermelon	11	20	21	28	3	7	3	7
Pineapple	15	15	11	11	0	3	3	3
Tiger Nut	28	15	17	14	7	3	3	3

A, B, C, D represent the Restaurants

4. DISCUSSION

In this study, the processed fruit juices assessed showed high microbial load (Tables 1-4; Figs. 1-2). Bacteria isolated during the course of the study are *E. coli*, *Bacillus* spp, *Klebsiella* spp, *Streptococcus* spp and *Staphylococcus* spp. *Bacillus* spp. was the most isolated bacterium during the course of the research, followed by *Staphylococcus* spp. However, *Streptococcus* spp. was the least isolated bacterium. According to the results, *Bacillus* spp. had the percentage of 31.4%, while the percentage of *Staphylococcus* spp. was 20.1% (Fig. 2). These results are similar to the findings of researchers who carried out their research on Orange, Watermelon and Pineapple juices in Port Harcourt, Nigeria. In their study, they recovered *Staphylococcus aureus* (28%); *Bacillus cereus* (26%); *Klebsiella pneumonia* (14%); *Escherichia coli* (5%); *Salmonella enteric*a (4%) and *Shigella* spp. (3%) [4]. Nma et al. [4] also isolated some fungi and they were identified as *Penicillium* sp (8%), *Aspergillu*s sp. (5%), *Fusarium* sp. (4%) and *Mucor* (3%). The results from this present study are also in line with the works of researchers who carried out their researches in other parts of Nigeria. These researchers reported the presence of bacteria namely, *Bacillus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Micrococcus* spp. and *Corynebacterium* spp. [8,9]. The presence of some of these bacteria (*Staphylococcus* spp. and *Bacillus* spp.) could indicate health risk to the consumers because they produce potent toxins associated with food borne illnesses [13].

Among the fruit juices, Tiger nut juice had the highest number of *Bacillus* spp., followed by Watermelon. Although, previous researchers have reported that Pineapple juice recorded high level of heterotrophic plate count (ranged from 3.5×10^4 to 6.1×10^4 CFU/ml). The same researchers reported that more bacteria count was observed in Pineapple than in Watermelon and they stated that it is because the ability of microorganisms to grow on the juices was largely attributed to less inhibitory nature of Watermelon juices owing to the higher P^H value (5.2) [4]. In addition, in India some researchers reported that microbial load in orange juice was comparatively higher than that in Pineapple [14]; this is not in line with the reports from the present study. The differences in the results could be as a result of poor and inappropriate handling and storage of the fruits before processing them.

Results of the heterotrophic plate count (HPC) ranged from 2.7×10^2 to 7.1×10^3. Results according to restaurants showed that Restaurant C had the highest HPC (7.1×10^3) from Tiger nut, while restaurant B had the lowest HPC (2.7×10^2) from Orange juice (Table 3). However, Restaurants A and B had the highest total coliform counts (28) from Tiger nut and Watermelon, respectively. Also, from this study, the total *E. coli* count according to most probable number MPN) technique showed that *E. coli* was more in fruit juices isolated from Restaurant D. Generally, the HPC of all the fruit juices exceeded the standard recommended limits by the [15]. This result is in accordance with the findings of other researchers who recorded the presence of *E. coli* in fruit juices [5,4,10,16]. The presence of *E. coli* and other coliforms portends unsanitary conditions resulting from unhygienic practices during or after processing of the fruit juices.

The recommended microbiological standard for any fruit juice as recommended by [17] is as follows: the minimum bacterial load anticipated for total viable (HPC) is 5.0×10^3, coliform is 10 and faecal coliform (*E. coli*) is 0. However, the maximum bacterial load permitted is 1.0×10^4 (total viable count or HPC), coliform is 100 and faecal coliform is 0. Also, according to [18] UNBS, 2009, the microbiological limits in fruit juices is maximum of 10^3 CFU/g total plate count. Generally, according to this study, most of the fruit juices were not favourable for consumption. This is because all the fruit juices collected from the four restaurants harboured faecal coliform (*E. coli*). According to Andres et al. [19], the presence of coliform (*E. coli*) in fruit juices is not allowed safe food consumption standard. Its presence in the fruit juices is of public health importance. Therefore, Pascalization, which is the method of preserving and sterilizing food, in which a product is processed under very high pressure is required. This method of Pascalization leads to the inactivation of certain microorganisms (including *E. coli*) and enzymes in the food [20].

The presence of microbial contaminants in all the products could be a reflection of the quality of the raw materials, processing equipment, environment, packaging materials, storage conditions and the personnel's involved in the production and distribution processes. Regard-less of the nature and origin of fruit juices, they should not be stored for a long time as prolonged storage could encourage spoilage when the fruit juices are not pasteurized [21,22].

5. CONCLUSION

Although, freshly produced fruit juices are very nutritious, the consumption of such juices could increase the risk of foodborne infections such as salmonellosis and gastrointestinal infection caused by pathogenic microorganisms. This is because of the different bacteria isolated in this study, including *E. coli*. *Escherichia coli.*

The unhygienic process of making the fruit juices, including the utensils and equipment used could contribute to the microbial load as observed in the present study. Hence, Pascalization of the fruit juices after processing is important. This method of sterilization is capable of inactivating *E. coli,* which is an indicator of faecal contamination. It is also important to educate different restaurants and eateries, where fresh fruit juices are sold of the necessity to practice good hygiene and Pascalize fruit juices before they are sold to consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57158