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Assessment of Microorganisms, Virulence Factors and Heavy Metals in Fresh Fruit Juices

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ABSTRACT

Fresh fruit juice consumption has become very popular because of its health benefits. But there's growing concern about their microbiological safety, particularly in cities like Port Harcourt. This study investigated microorganisms and heavy metals associated with fresh fruit juices sold in Port Harcourt. A total of one hundred and twenty (120) fresh fruit juices: orange, watermelon, Tiger nut and pineapple juices were sampled from ten different vendors in Port Harcourt. The microbial load and identification were determined using standard microbiological methods. The pH, Lead, Mercury and Arsenic of the juices were determined using standard method. The mean range in colony forming unit per milliliter (CFU/ml) of total heterotrophic bacterial load of the fruits was: 0.74±0.7 to 2.8±0.3×10^6 CFU/mL. Faecal coliform was 0.10±0.8 to 9.8±0.1×10^3 CFU/mL. Coliform load was 0.62±0.4 to 2.7±0.5×10^5, CFU/mL. *Salmonella* load was 0.010±0.1-11.0×10^4 CFU/mL while the count of *Shigella* was 0.25±0.3 - 10.6×10^3 CFU/mL, respectively. The highest bacterial load was observed in the watermelon juice while the least was in orange juice. Eight bacterial genera: *Staphylococcus*, *Serratia*, *Bacillus*, *Escherichia*, *Salmonella*, *Vibrio*, *Shigella* and *Flavobacterium* sp were isolated from the juices. Virulence results showed that 28.6-100 % of the isolates produced biofilm, 14.3-66.7% were lecithinase positive, 20-85.7% was haemolytic, 11.1-100% produced hydrogen suphide, while 85.7% of Staphylococcus sp were coagulase positive. The pH ranged from 4.7-6.65, while the Lead, Mercury and Arsenic in the juices ranged from 0.03-2.9mg/ml, 0.073-0.819mg/l and 0.0205-0.56 mg/ml, respectively. The presence of suspected pathogens and high levels of heavy metals could cause health challenges to consumers, especially to those with weak immune systems.

Keywords: Fresh fruit juice, microorganisms, virulence factors, heavy metals, microbiological safety.

Introduction

Fruit juices are rich in nutrients, minerals, and vitamins, and have a fleshy flavour that is beneficial to health (Wedajo and Kadire, 2019). Fruit juice is nutritious and plays a crucial role in a healthy diet because it offers a variety of micronutrients found in earth (Nelofer *et al*., 2015). Fruit juices are well appreciated by consumers because of their taste, nutritional value and availability at the right time. They are also an important part of the modern diet in many countries (Raybaudi-Massilia *et al.,* 2009). The demand for fresh cut – fruit and unpasteurized fruit juices have increased in the last decades, due to the content of antioxidants, vitamins and minerals that these foods can supply to man, which play important roles in the prevention of heart disease, cancer and diabetes (Matthews, 2006).

Nonetheless, fresh fruit juices are highly susceptible to spoilage, since fluid contents (enzymes, organic acids and carbohydrates, etc) are in contact with air and microorganisms from the environment while handling. Thus, if juice is not rapidly heated a fast microbial, enzymatic, chemical and physical deterioration takes place and a shorter shelf life is observed (Bates *et al*., 2001). Despite the potential benefits offered, concerns over their safety and quality have been raised. There is now increasing concern about pathogenic microorganisms among regulators regarding the safety of juices due to the potential ability of these pathogens to survive during the manufacturing process. Fruit juices contain microflora which is normally present on the surface of fruits during harvest and postharvest processing which include transport, storage and processing (Tournas *et al*., 2006).

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Many microorganisms such as acid tolerant bacteria and fungi (moulds, yeasts) use them as a substrate for their growth. Among bacteria, lactic acid bacteria and acetic acid bacteria have been isolated from fruit juices (ICMSF, 2005). Major microorganisms commonly found in street juice include *Escherichia coli*, *Salmonella typhi*, *Pseudomonas* spp, *Staphylococcus aureus* and *Vibrio cholerae*. These pathogens are linked to typhoid fever, food poisoning, gastroenteritis, enteric fever and diarrhea, which, in many cases, become life threatening across the globe (Aneja *et al.*, 2014). Aside the public health significance of microorganisms in fruit juices, heavy metals in these juices is a vital parameter to consider especially with its implication in the health of man. This study therefore assesses the microorganisms, virulence factors and heavy metals found in unpasteurized fresh fruit juices sold in Port Harcourt metropolis, Rivers State.

Materials and Methods

Sample Collection

A total of one hundred and twenty (120) locally produced fresh fruit juice samples: orange, watermelon, Tiger nut and pineapple juices were bought from ten (10) different local fruit juice vendors in Port Harcourt. Commercially pasteurized fruit juices were bought and used as a positive control. Four different juices were bought from each local fruit juice vendor. Thus, 40 juices were bought monthly for three months making a total of 120 samples. The samples which were sealed in plastic containers by the local fruit vendors placed in ice-packed containers and transported to the Microbiology laboratory, Department of Microbiology Rivers State University for immediate analysis. The map of the sampled locations is shown in Figure 1.

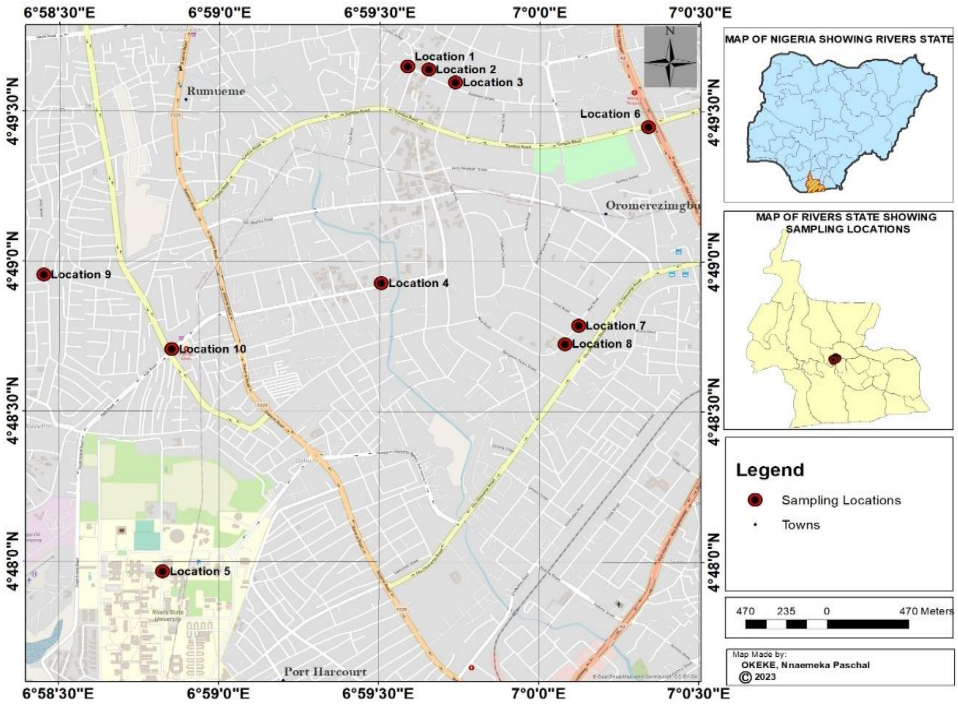

Fig. 1: Map of the Locations where fruit Juice samples were bought

Enumeration and Isolation of Microorganisms

Stock solution of the fruit juices were first prepared by transferring 1mL of the juice sample with the aid of a sterile pipette into a test tube containing 9mL sterile normal saline. Subsequent dilution was followed serially by transferring 1mL from the stock into another test tube containing sterile 9mL of normal saline. This was continued until a dilution of 1:10, 000 (10^{-4}) was achieved. Aliquots (0.2mL) of 10^{-2} and 10^{-3} were inoculated on McConkey agar, mannitol salt agar, Eosin methylene blue agar, *Salmonella-Shigella* agar and thio citrate bile sucrose agar plates for enumeration and isolation of coliform, staphylococci, faecal coliform *Salmonella-Shigella* and *Vibrio*, respectively.

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While 0.2mL of 10^{-4} dilution was inoculated on nutrient agar plates for enumeration and isolation of the total heterotrophic bacteria. Inoculated plates were spread using sterile bent glass rod and incubated at 37ºC for 24-48 hours while faecal plates for coliforms were incubated at 44ºC for 24-48 hours.

After incubation, colonies on respective plates were counted and discrete colonies were subcultured onto freshly prepared pre-dried nutrient agar. Pure cultures were isolated and identified using morphological and biochemical tests (indole, methyl red, Voges Proskauer, sugar fermentations, citrate utilization, motility and H_2S tests) (Cheesbrough, 2006).

Phenotypic Determination of Virulence Factors of the Bacterial Isolates

Coagulase Test

This test was used to determine whether coagulase was present (an enzyme that coagulates blood). It helps to distinguish between *Staphylococcus epidermidis* and pathogenic *Staphylococcus aureus*. On clean grease-free microscope slides, a colony of the test isolate was emulsified on a drop of saline on one end while the other end had only saline. After which, a drop of human plasma was applied on both ends. Both ends were observed immediately for clumping. Coagulase-positive *S. aureus* would clump after 10-15 seconds while non-coagulase-positive *Staphylococcus* sp would not clump (Robinson *et al.*, 2023).

Lecithinase Test

The purpose of this experiment was to determine whether the isolates could create the enzyme lecithinase, also known as α-toxin, which combines with lecithin in egg-yolk medium to form an iridescent layer that denotes lypolysis and an opalescence that shows lecithinase activity. The isolates were applied to the egg yolk medium in a single line by streaking with a sterile wire loop.

The plates were incubated at 37 ºC for 24 hours and examined for opalescent halo surrounding the inoculum inoculum. The appearance of a white, opaque, diffuse zone that extends into the medium surrounding the colonies indicated a positive test while the absence of a white, opaque zone extending from the edge of the colony signified a negative test (Robinson *et al.*, 2023).

Haemolysis Test

Investigations were made into the isolates' hemolytic activity. This was done to determine whether the bacterial isolates could break down red blood cells. By streaking the isolates onto aqueously made blood agar medium according to Sagars (2015). The plates incubated at 37℃ for 24-48 hours after which the plates were read for the presence of beta (complete zone of inhibition), gamma (no haemolysis) or alpha (partial clearing of zones) haemolysis (Robinson *et al.*, 2023).

Biofilm Test

This was done as described by Robinson *et al.*, (2023). Congo red agar (CRA) is the solid media used in the CRA plate test. With the use of this technique, it is possible to directly analyse the colonies and distinguish between slime-forming strains (which show up as black colonies on red agar) and non-slime-forming strains (red-coloured colonies). The strains that pass the test have red colonies with unchanging colour and black spikes on them. As a result, bacterial isolates were grown on CRA plates that were made by mixing 1 L of Brain Hart Infusion agar (BHI) with 0.8 g of Congo red and 36 g of saccharose (both from Sigma, Missouri, EUA) (Oxoid, Basingstoke, Hampshire, England). After that, the plates were incubated for 24 hours at 37°C. Slime-producing strains were distinguished from non-slime-producing isolates (red smooth colonies) by the presence of rough black colonies (de Castro Melo *et al.*, 2013).

Heavy Metal Analysis of Fresh Fruit Samples

Analysis of Heavy metals Lead (Pb), Mercury (Hg) and Arsenic (Ar) of the fruit samples were done by using atomic absorption spectroscopy (Sriadibhatla, 2013).

Statistical Analysis

The mean and standard deviation of the microbial counts was determined using descriptive statistics. Two-way analysis of variance was carried out to check for significant difference. The significant level was adjusted to $P<0.05$, thus, cases that showed significant differences, the Duncan multiple range test was used in mean separation. All Analysis was done using the Statistical Package for Social Science software (SPSS version 27).

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Results

Results of the microbial load of orange fruit juices are as shown in Table 1. The mean range of the total heterotrophic bacterial, faecal coliform, *Salmonella, Shigella,* total coliform, total *Vibrio* and fungal load of the orange juices was 0.74±0.7 - 2.6±0.5 $\times10^6$, 0.10±0.8-8.0±0.7$\times10^3$, 0.010±0.1-11.0$\times10^4$, 0.25±0.3-5.8±0.3$\times10^3$, 0.62±0.4-2.7±0.5$\times10^5$, 0.0±0.0-7.5±0.7$\times10^2$ and 0.21±0.7-2.3±0.2$\times10^5$ CFU/ml, respectively (Table 1).

The results of the microbial load of the Pineapple fruit juices are as shown in Table 2. The mean range of total heterotrophic bacterial, faecal coliform, *Salmonella, Shigella,* total coliform, total *Vibrio* and fungal load of the pineapple juices was 1.0±0.2-2.7±0.2$\times10^6$, 1.3±0.1-9.3±1.1$\times10^3$, 0.65±0.07-6.1±3.2$\times10^3$, 1.1±0.8-8.0±0.9$\times10^3$, 0.90±0.5-2.6±0.4$\times10^5$, 0.0±0.0-3.8±0.5$\times10^3$ and 0.52±0.4-2.0±0.2$\times10^5$ CFU/ml, respectively (Table 2).

Table 1: Mean Microbial Load (CFU/ml) of Orange Juice in the various Locations

Juice Samples	THB ($\times10^6$)	TFC ($\times10^3$)	SA ($\times10^3$)	SH ($\times10^3$)	TC ($\times10^5$)	TV ($\times10^2$)	FC ($\times10^4$)
OL1	1.6±0.8^{ab}	7.8±0.2^{b}	0.25±0.3^{a}	4.8±0.3^{bc}	1.4±0.1^{ab}	0.00±0.0^{a}	2.2±0.1^{a}
OL 2	0.86±0.3^{ab}	5.5±0.4^{ab}	5.1±0.4^{b}	5.0±0.5^{bc}	1.9±0.2^{bc}	0.12±0.1^{a}	2.1±0.7^{a}
OL 3	1.3±0.8^{ab}	6.5±0.3^{ab}	1.5±0.5^{a}	3.2±0.2^{abc}	2.7±0.1^{c}	0.00±0.0^{a}	15.5±0.1^{bc}
OL 4	2.6±0.5^{c}	8.0±0.7^{b}	11.0±0.4^{c}	2.4±0.1^{abc}	2.7±0.5^{c}	0.31±0.3^{a}	3.8±0.2^{a}
OL 5	0.74±0.7^{a}	7.8±02^{b}	0.10±0.4^{a}	2.9±0.2^{abc}	1.9±0.1^{bc}	0.00±0.0^{a}	2.4±0.2^{a}
OL 6	0.97±0.1^{ab}	0.36±0.4^{ab}	0.67±0.5^{a}	0.95±0.5^{ab}	1.1±0.5^{ab}	0.35±0.4^{a}	4.0±0.2^{a}
OL 7	1.3±0.9^{ab}	0.11±0.1^{a}	0.25±03^{a}	0.77±0.8^{ab}	0.62±0.4^{a}	0.25±0.5^{a}	5.7±0.4^{ab}
OL 8	1.8±0.6^{b}	2.5±0.2^{a}	0.10±0.1^{a}	5.8±0.3^{c}	1.3±0.4^{ab}	7.5±0.7^{b}	23.2±0.2^{c}
OL 9	0.95±0.3^{ab}	0.44±0.5^{a}	0.60±0.5^{a}	1.4±0.1^{ab}	1.2±0.9^{ab}	0.25±0.5^{a}	10.8±0.3^{ab}
OL 10	1.5±0.6^{ab}	0.10±0.8^{a}	0.10±0.7^{a}	0.25±0.3^{a}	1.0±0.3^{ab}	0.00±0.0^{a}	10.5±0.9^{ab}
Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
P-value	0.004	0.05	0.001	0.05	0.00	0.02	0.001

*Means with similar superscript down the group showed no significant difference (*P> 0.05*)
Keys: THB-Total Heterotrophic Bacteria count, TFC-Total faecal coliform count, SA-*Salmonella* count, SH-*Shigella* count, TC-total coliform count, TV-Total Vibrio count, FC-fungal count, L-location, O-orange juice

Table 2: Mean Microbial Load (CFU/ml) of Pineapple Juice in the various Locations

Juice Samples	THB ($\times10^6$)	TFC ($\times10^3$)	SA ($\times10^3$)	SH ($\times10^3$)	TC ($\times10^5$)	TV ($\times10^2$)	FC ($\times10^5$)
PIN L1	1.7±0.7^{ab}	5.7±0.6^{a}	1.7±0.2^{a}	5.4±0.2^{bc}	1.3±0.7^{ab}	2.5±0.0^{a}	1.4±0.5^{ab}
PIN L2	2.7±0.2^{c}	1.7±0.2^{a}	3.1±0.3^{ab}	8.0±0.9^{d}	2.6±0.4^{d}	0.0±0.0^{a}	0.57±0.4^{ab}
PIN L3	1.7±0.2^{ab}	9.3±1.1^{a}	2.8±1.8^{ab}	2.3±1.7^{a}	2.4±0.6^{cd}	38.5±0.5^{b}	1.6±1.4^{abc}
PIN L4	1.0±0.4^{a}	3.3±2.6^{a}	6.1±3.2^{b}	6.8±0.4^{cd}	2.1±0.3^{bcd}	0.0±0.0^{a}	0.43±0.3^{a}
PIN L5	1.5±1.2^{ab}	2.9±2.6^{a}	0.65±0.07^{a}	3.5±1.3^{ab}	1.5±1.1^{abc}	0.0±0.0^{a}	0.52±0.4^{ab}
PIN L6	1.1±0.5^{a}	5.1±0.5^{a}	3.6±0.4^{ab}	1.5±1.1^{a}	1.2±1.0^{ab}	3.5±0.4^{a}	1.6±0.5^{abc}
PIN L7	1.5±0.5^{ab}	4.8±0.5^{a}	0.65±0.07^{a}	2.6±1.0^{a}	1.2±0.6^{ab}	7.5±0.5^{a}	2.0±0.2^{c}
PIN L8	1.4±0.8^{a}	4.5±1.9^{a}	1.3±0.1^{a}	5.1±3.8^{bc}	1.4±0.6^{abc}	3.8±1.7^{a}	1.8±1.1^{bc}
PIN L9	1.4±0.9^{a}	1.3±0.1^{a}	1.9±0.7^{a}	1.6±0.9^{a}	1.1±0.2^{ab}	2.5±0.0^{a}	1.8±1.0^{abc}
PIN L10	1.0±0.2^{a}	3.3±2.3^{a}	1.1±0.07^{a}	1.1±0.8^{a}	0.90±0.5^{a}	1.7±0.5^{a}	1.0±0.08^{abc}
Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
P-value	0.05	0.55	0.03	0.00	0.01	0.02	0.05

*Means with similar superscript down the group showed no significant difference (*P> 0.05*)
Keys: THB-Total Heterotrophic Bacteria count, TFC-Total faecal coliform count, SA-*Salmonella* count, SH-*Shigella* count, TC-total coliform, TV-Total Vibrio count, FC-fungal count, L-location, PIN-pineapple juice

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Results of the microbial load of Tiger nut fruit juices showed that the mean range of the total heterotrophic bacterial, faecal coliform, *Salmonella, Shigella*, total coliform, total *Vibrio* and fungal load of the Tiger nut juices was 1.1±0.2-2.1±0.5×10^6, 1.1±0.1-9.8±0.1×10^3, 0.65±0.2-5.2±0.3×10^3, 1.2±0.6-10.6×10^3, 1.1±0.4-2.6±0.5×10^5, 0.0±0.0-2.9±0.3×10^3 and 1.1±0.2-9.9±0.1×10^4 CFU/ml, respectively (Table 3).

Results of the microbial load of watermelon juices showed that the mean range of the total heterotrophic bacterial, faecal coliform, *Salmonella, Shigella*, total coliform, total *Vibrio* and fungal load of the watermelon juices was 1.4±0.4-2.8±0.3×10^6, 1.2±0.4-8.3±0.9×10^3, 0.031±0.3-1.0±0.5×10^4, 0.30±0.1-1.0±0.8×10^4, 1.1±0.1-2.5±0.4×10^5, 0.0±0.0-1.3±0.1×10^4 and 1.3±0.1- 1.7±0.1×10^5 CFU/ml, respectively (Table 4). More so, findings showed that the total heterotrophic bacterial, faecal coliform, *Salmonella* and total coliform load of orange juices obtained from location 4 was significantly (P<0.05) higher than counts from similar microbial parameters in other locations.

The *Shigella, Vibrio* and fungal load of oranges in location 8 was significantly (P<0.05) higher than similar parameters of other locations. The total heterotrophic bacterial load, *Shigella* load and total coliform load of pineapple juices from location 2 was significantly (P<0.05) higher than the counts of similar microbial parameters obtained from other locations in pineapple juices. Faecal coliform load of pineapple juices showed no significant difference (P>0.05) despite the high counts recorded in location 3 and 1. There were no *Vibrio* counts detected in locations 2, 4 and 5, respectively, while *Vibrio* counts recorded in location 3 was significantly (P<0.05) higher than counts obtained in other locations.

Findings showed that the total heterotrophic bacterial load and *Salmonella* load of the Tiger nut juices in locations 3 was significantly (P<0.05) higher than similar parameters of Tiger nut juices from other locations. There were no significant differences (P>0.05) in the faecal coliform load, *Vibrio* and fungal loads of the Tiger nut juices in the different locations whereas the *Shigella* and total coliform load of Tiger nut in locations 4 was significantly (P<0.05) higher than counts obtained from other Tiger nut juices in other locations.

Results of the morphology, biochemical and probable identity of the bacterial isolates showed that the bacterial isolates belonged to eight genera: *Staphylococcus, Serratia, Vibrio, Bacillus, Escherichia, Salmonella, Shigella* and *Flavobacterium*.

Table 3: Mean Counts of the Microbial Load (CFU/ml) of Tiger Nut Juice in the various Locations

Juice Samples	THB (×10^6)	TFC (×10^3)	SA (×10^3)	SH (×10^3)	TC (×10^5)	TV (×10^3)	FC (×10^4)
TNL1	1.3±0.2 ^{ab}	9.8±0.1 ^{a}	0.87±0.7 ^{a}	1.9±0.2 ^{a}	2.0±0.9 ^{bcd}	0.00±0.0 ^{a}	2.3±0.4 ^{a}
TNL2	2.0±0.2 ^{bc}	1.1±0.1 ^{a}	4.7±0.2 ^{bc}	4.6±0.2 ^{ab}	2.4±0.3 ^{d}	1.4±0.1 ^{a}	6.3±0.4 ^{a}
TNL3	2.1±0.5 ^{c}	7.6±0.8 ^{a}	5.2±0.3 ^{c}	5.6±0.5 ^{ab}	2.4±0.3 ^{cd}	2.1±0.2 ^{a}	4.9±0.3 ^{a}
TNL4	1.6±0.5 ^{abc}	9.7±0.9 ^{a}	2.7±0.1 ^{abc}	10.6±0.2 ^{c}	2.5±0.1 ^{d}	1.1±0.1 ^{a}	1.1±0.2 ^{a}
TNL5	1.4±0.5 ^{abc}	4.9±0.5 ^{a}	3.1±0.4 ^{abc}	7.3±0.4 ^{bc}	2.1±0.9 ^{bcd}	2.9±0.3 ^{a}	8.8±0.8 ^{a}
TNL 6	1.1±0.2^{a}	8.0±0.8 ^{a}	2.6±0.2 ^{abc}	5.5±0.2 ^{ab}	1.6±0.6 ^{ab}	1.3±0.1 ^{a}	4.9±0.4 ^{a}
TNL7	1.1±0.7 ^{a}	7.5±0.5 ^{a}	2.8±0.3 ^{abc}	5.0±0.5 ^{ab}	1.1±0.4 ^{a}	0.25±0.2 ^{a}	7.9±0.6 ^{a}
TNL8	1.4±0.7 ^{abc}	7.7±0.8 ^{a}	1.6±0.2 ^{ab}	2.2±0.1 ^{a}	2.6±0.5 ^{d}	3.2±0.3 ^{a}	1.2±0.1 ^{a}
TNL9	1.99±0.1 ^{abc}	4.2±0.4 ^{a}	0.65±0.2 ^{a}	3.3±0.2 ^{ab}	1.5±0.7 ^{ab}	0.92±0.8 ^{a}	4.6±0.3 ^{a}
TNL10	1.5±0.6^{abc}	5.4±0.6 ^{a}	0.65±0.6 ^{a}	1.2±0.6 ^{a}	1.7±0.1 ^{abc}	0.25±0.1 ^{a}	9.9±0.1 ^{a}
Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
p-value	0.05	0.96	0.04	0.02	0.00	0.27	0.64

*Means with similar superscript down the group showed no significant difference (*P> 0.05*)

Keys: THB-Total Heterotrophic Bacteria count, TFC-Total faecal coliform count, SA-*Salmonella* count, SH-*Shigella* count, TC-total coliform, TV-Total Vibrio count, FC-fungal count, L-location, TN-tiger nut juice,

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Table 4: Mean Microbial Load (CFU/ml) of Watermelon Juice in the various Locations

Juice Samples	THB ($\times10^6$)	TFC ($\times10^3$)	SA ($\times10^3$)	SH ($\times10^3$)	TC ($\times10^5$)	TV ($\times10^2$)	FC ($\times10^4$)
WML1	2.8±0.3^{c}	8.3±0.9^{b}	10.2±0.5^{d}	8.9±0.6^{b}	2.5±0.4^{b}	13.1±0.1^{a}	12.5±0.1^{b}
WML2	1.6±0.2^{a}	2.5±0.2^{ab}	7.1±0.1^{cd}	10.0±0.8^{b}	1.3±0.3^{b}	0.00±0.0^{a}	4.3±0.3^{a}
WML3	1.8±0.3^{ab}	3.5±0.4^{ab}	4.1±0.2^{abc}	5.9±0.1^{ab}	2.3±0.4^{b}	0.25±0.5^{a}	11.4±0.1^{a}
WML4	1.8±0.9^{ab}	3.0±0.3^{ab}	3.8±0.1^{abc}	8.7±0.8^{b}	2.4±0.3^{b}	0.55±0.6^{a}	1.3±0.1^{a}
WML5	1.4±0.4^{a}	1.5±0.1^{a}	5.1±0.6^{bc}	6.0±0.5^{ab}	2.2±0.1^{b}	0.12±0.1^{a}	13.5±0.3^{b}
WML6	2.3±0.3^{bc}	2.3±0.2^{ab}	0.57±0.5^{a}	5.4±0.1^{ab}	2.3±0.3^{b}	9.5±0.9^{a}	5.9±0.2^{a}
WML7	2.3±0.2^{bc}	1.6±0.1^{a}	0.42±0.4^{a}	2.0±0.3^{a}	1.7±0.7^{ab}	130.7±0.1^{b}	17.2±0.1^{c}
WML8	2.6±0.2^{c}	1.4±0.1^{a}	0.92±0.8^{a}	5.0±0.2^{ab}	1.7±0.7^{ab}	31.7±0.3^{a}	8.7±0.6^{a}
WML9	1.5±0.3^{a}	5.2±0.4^{ab}	1.1±0.1^{a}	3.1±0.1^{a}	1.1±0.1^{a}	1.2±0.5^{a}	3.9±0.2^{a}
WML10	1.7±0.3^{ab}	1.2±0.4^{a}	0.31±0.3	3.0±0.1^{a}	1.8±0.8^{ab}	0.75±0.9^{a}	5.9±0.4^{a}
Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
p-value	0.00	0.03	0.00	0.03	0.01	0.02	0.3

*Means with similar superscript down the group showed no significant difference ($P> 0.05$)

Keys: THB-Total Heterotrophic Bacteria count, TFC-Total faecal coliform count, SA-*Salmonella* count, SH-*Shigella* count, TC-total coliform, TV-Total Vibrio count, FC-fungal count, L-location, WM-watermelon juice

Results of the virulence test of the bacterial isolates presented in Table 5 showed that 57.1% of *Staphylococcus* sp were positive for biofilm production, 42.9% had the lecithinase enzyme while 85.7% were positive for haemolysis and coagulase. For *E. coli* isolates, 62.5, 25 and 37.5% were positive for biofilm production, lecithinase and hydrogen sulfide production while 66.7%, 27.8, 66.7 and 100% *Salmonella* sp were positive for biofilm production, lecithinase, haemolysis and hydrogen sulfide production. *Shigella* sp (41.7%) and *Serratia* sp (100%) were only positive for biofilm production.

While 28.6, 14.3, 28.6 and 42.9% *Vibrio* isolates were positive for biofilm, lecithinase, haemolysis and hydrogen sulfide production. Results further showed that no *Flavobacterium* isolates produced hydrogen sulfide. For the *Bacillus* isolates, 33.3, 66.7, 44.4 and 11.1% produced biofilm, lecithinase, haemolysis and hydrogen sulfide, respectively.

Results of the heavy metal analysis of the fruit juices in Table 6 showed that the pH ranged between 4.7 and 6.65, while Lead, Mercury and Arsenic in the juices ranged from 0.03 to 2.9mg/ml, 0.073 to 0.819mg/l and 0.0205 to 0.56 mg/ml, respectively.

Table 5: Virulence factors of bacteria isolated from all the types of fruit juices

Isolate	No. of juice samples	% Biofilm	Lecithinase (%+ve)	Haemolysis (%+ve)	H_2S	Coagulase
Staphylococcus sp	7	4 (57.1)	3 (42.9)	6 (85.7)	0	6 (85.7)
E. coli	16	10 (62.5)	4 (25)	0	6 (37.5)	NA
Salmonella sp	18	12 (66.7)	5 (27.8)	12 (66.7)	18 (100)	NA
Shigella sp	12	5 (41.7)	0	0	0	NA
Vibrio sp	7	2 (28.6)	1 (14.3)	2 (28.6)	3 (42.9)	NA
Bacillus sp	9	3 (33.3)	6 (66.7)	4 (44.4)	1 (11.1)	NA
Flavobacterium sp	10	3 (30)	6 (60)	2 (20)	0	NA
Serratia sp	1	1 (100)	0	0	0	NA

Keys: +ve = positive, % = percentage, NA = not applicable

Citation: George *et al*. (2024). Assessment of microorganisms, virulence factors and heavy metals in fresh fruit juice. *International Journal of Microbiology and Applied Sciences*. *3(1)*: 91 – 100.

Table 6: Heavy Metal Concentration of the Fruit Juices

Location	pH	Lead (mg/l)	Mercury (Hg) (mg/l)	Arsenic (As) (mg/l)
OL1	5.75±0.1	0.598±0.0	0.239±0.0	0.1505±0.0
OL2	5.5±0.0	0.5225±0.0	0.2805±0.0	0.1095±0.0
OL3	6±0.0	1.82±0.0	0.3205±0.0	0.3605±0.0
OL4	5.65±0.1	2.913±0.0	0.3295±0.0	0.2195±0.0
OL5	5.65±0.1	1.9235±0.0	0.231±0.0	0.121±0.0
OL6	5.5±0.0	2.475±0.3	0.8195±0.0	0.271±0.0
OL7	5.9±0.1	1.8205±0.0	0.5505±0.0	0.159±0.1
OL8	6±0.0	0.8195±0.0	0.7295±0.0	0.0705±0.0
OL9	5.95±0.1	0.6105±0.0	0.231±0.0	0.2215±0.0
OL10	6±0.0	0.614±0.0	0.3135±0.0	0.142±0.0
PINL1	5.6±0.1	0.708±0.0	0.0805±0.0	0.3305±0.0
PINL2	5.3±0.0	0.6465±0.0	0.1995±0.0	0.23±0.0
PINL3	4.85±0.1	0.192±0.0	0.1005±0.0	0.4805±0.0
PINL4	5.65±0.1	0.033±0.0	0.1895±0.0	0.1495±0.0
PINL5	5.45±0.1	0.076±0.0	0.2305±0.0	0.2505±0.0
PINL6	4.7±0.0	0.7205±0.0	0.1295±0.0	0.0205±0.0
PINL7	6.5±0.1	0.72±0.0	0.1905±0.0	0.2195±0.0
PINL8	6.05±0.1	1.6605±0.0	0.2195±0.0	0.1105±0.0
PINL9	5.8±0.0	0.271±0.0	0.18±0.0	0.1305±0.0
PINL10	5.95±0.1	0.2075±0,0	0.073±0.0	0.2105±0.0
WML1	6.1±0.1	2.3105±0.0	0.3705±0.0	0.8705±0.0
WML2	6.05±0.1	2.5205±0.0	0.3395±0.0	0.56±0.0
WML3	6.3±0.0	2.4095±0.0	0.4405±0.0	0.4495±0.0
WML4	6.5±0.1	2.2765±0.0	0.4805±0.0	0.2405±0.0
WML5	6.05±0.1	1.6545±0.0	0.44±0.0	0.3205±0.0
WML6	6.55±0.1	0.7905±0.0	0.3205±0.0	0.1905±0.0
WML7	6.65±0.1	0.73±0.0	0.4295±0.0	0.1305±0.0
WML8	6.4±0.1	0.7705±0.0	0.62±0.0	0.2±0.0
WML9	6.45±0.1	0.7195±0.0	0.8305±0.0	0.1305±0.0
WML10	6.6±0.0	0.6295±0.0	0.6195±0.0	0.17±0.0
WHO Limits	2.5-4.0	0.01	-	-

Keys: WM: water melon, PIN: pineapple, O: orange, L: locations

Discussion

There are lots of vendors who process fresh juices and retail them to consumers in Port Harcourt metropolis. The findings of this study showed very high bacterial load. The microbial load of the unpasteurized fruit juices was higher than the microbial loads of the pasteurized fruit juices in the present study. Bikila and Kadire (2019) reported that commercially available fruit juices have little or no microbial load compared to unpasteurized freshly made juices and this could be due to the pasteurization treatment and stringent manufacturing practices carried out on commercially available juices.

More so, the bacterial loads in the present study were higher than those reported by Odu *et al*. (2017) of fresh fruit juices sold in Port Harcourt. Although similar studies in other part of the world have documented high microbial loads in unpasteurized fruit juices which corroborates the present study (Aneja *et al.*, 2014; Babalola *et al.*, 2011). The total heterotrophic bacterial and coliform load of the orange, watermelon, Tiger nut and pineapple juices were generally high and exceeded the $<1.0\times10^3$ CFU/ml and $<1.0\times10^2$ CFU/ml specification of the International Commission for Microbiological Specification for Food (ICMSF, 2005.

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The counts were also higher than counts reported by Doyle (2010). Thus, the high microbial load observed in the present study varied across the type of fruit juices, the location of production of juices and this could be due to contamination from the environment, material used in processing and other factors such as experience in food processing and knowledge of food hazards adopted during fruit processing. Oranusi *et al.*, (2012) attributed the presence of microbiological contamination in fruit juices to poor raw materials, processing tools, environmental conditions, packing materials, and personnel in the production process while Odu *et al.*, (2017) attributed poor hygiene, use of contaminated water and poor storage conditions as reasons for microbial contaminations in the locally made fruit juices.

Out of the bacteria isolated, *Salmonella, Escherichia, Flavobacterium, Shigella, Vibrio, Staphylococcus,* and *Bacillus* spp. were isolated from orange juices; *Salmonella, Escherichia coli, Serratia, Shigella, Vibrio, Staphylococcus,* and *Bacillus* spp. were isolated from watermelon juice; *Salmonella, Escherichia coli, Shigella, Vibrio, Staphylococcus* and *Bacillus* spp were isolated from pineapple juice while *Salmonella, Escherichia coli, Vibrio, Staphylococcus* and *Bacillus* spp. were isolated from Tiger nut juice. The bacterial isolates in the unpasteurized fruit juices have been reported in previous studies. Odu *et al.* (2017) in a study of fresh fruit juices isolated four bacterial genera: *Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus* and *E. coli* which are similar with the present study except for the presence of *Klebsiella pneumoniae* which was not isolated in the present study. While they isolated four bacterial genera, this study isolated eight bacterial genera which was higher than those in the study of Odu *et al.* (2017). Oranusi *et al*. (2012) isolated *Lactobacillus* sp, *Enterobacter* sp, *Bacillus* sp, *Corynebacterium* sp and *S. aureus* which are also similar to the present study. The present study also showed that *Salmonella* sp was very prevalent in the various fruit juices. This agreed with De Jesús *et al.* (2022) who reported similar findings with the isolate having the highest prevalence amongst other isolates. The high nutrients in fruit juices could provide the required environment for microbial survival especially for pathogenic organisms such as *Salmonella, E. coli, Shigella* and other pathogens (Vantarakis *et al.*, 2011).

Shigella is a bacterial strain that can cause food poisoning. *Shigella* contamination of fruit juices can occur as a result of unsanitary raw material handling or contaminated water sources (Prescott *et al.*, 2011). *Salmonella* on the other hand is a common cause of foodborne illness. In fruit juices, contamination may occur through contaminated raw fruits or during processing (Rabsch *et al.*, 2001) while *Staphylococcus aureus* can produce toxins that cause food poisoning and consumption could lead to infections of the gastrointestinal tract.

Bacterial isolates in the present study have been reported to contain strains that could be pathogenic thereby causing diseases even at low doses of 10-100 cells (Kaczmarek *et al*., 2019). In agreement to the present study, Bikila and Kadire (2019) also reported lack of bacterial and fungal isolates in pasteurized fruit juices compared to the unpasteurized juices which had microbial growth that made the product risky for consumption.

The pH of the various fruits was within the acidic range. Fruits are known to have pH in acidic ranges. The pH of the various fruit juices varied across the locations. For instance, the pH of pineapple juice in location 6 was 4.7 while pH of pineapple juice in location 7 was 6.5. Acidic pH ranges in freshly processed fruit juices have been reported in previous studies. Odu *et al.* (2017) reported pH of 3.6, 3.5 and 5.2 for orange, pineapple and watermelon of freshly processed juice.

Although, these pH are within the acidic range but compared to the pH range of 4.7-6.5 of the present study, we could say theirs was more acidic. This could be the reason why the present study had higher bacterial load and bacterial genera than theirs as bacterial isolates do not thrive well at low pH ranges (Oranusi *et al.*, 2012).

The heavy metal concentrations in the present study were very high. Previous study reported 0 concentration of Lead, arsenic, copper and iron in fruit juices (Oranusi *et al.*, 2012). While regulations standards are based on regions, it is important to note that consumption of heavy metals could cause diseases such as cancers as well as failures in organ systems (Prescott *et al.*, 2011).

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The U.S. FDA has not established specific limits for mercury in fruit juices while the Codex Alimentarius Commission, which sets international food standards, has established maximum levels for total mercury in some seafood products, but specific limits for fruit juice do not exist (CAM, 2021).

The concentrations of lead in the samples were greater than the WHO permissible limit of 0.01 mg/L (WHO, 2011). This implied that the fruit juices were diluted with water contaminated with lead or the fruits may have been contaminated by heavy metals. Lead is linked with health effects such as cancer, brain damage, renal, endocrine and reproductive disorders (Vella *et al*., 2010). The lead concentration reported by Oranusi *et al.* (*2*012) ranged from 0.0427±0.0003 to 0.345 ±0.0003 mg/L in pineapple juice while the Lead concentration of pineapple juice in the present study ranged from 0.033±0.0 to 1.6605±0.0. Thus, the lead concentration in the present study is slightly higher than the range reported in their study.

Exposure to high levels of mercury, particularly methylmercury, can lead to neurological and developmental issues, especially in foetuses and young children. It can affect the nervous system and cognitive function while chronic exposure to elevated levels of arsenic could be associated with various health problems, including skin lesions, cardiovascular diseases, and an increased risk of certain cancers (Mozaffarian and Rimm, 2006; Hughes *et al.*, 2011). The presence of high levels of arsenic and mercury in these juices could be through the environment from which fruits were grown and harvested, the water and other materials used during processing.

In conclusion, this study revealed that the bacterial load in the fruit juices across the respective locations were very high and exceeded the tolerable or allowable limits. Some of the public health important bacterial isolates such as *S. aureus*, *Salmonella enterica*, *Shigella sonnei*, *Serratia marcescens* and *Vibrio cholera*e were isolated from the fruit juices. The presence of these isolates could pose serious public health concern especially as some of these isolates contained virulent strains. The levels of Lead, mercury and arsenic in the fruit juices were very high. Despite the absence of recommended limits for mercury and arsenic in fruit juices, their presence in food or water has been considered as threat since they could cause different neurological disorder amongst other diseases.

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