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Isolation, Characterisation and Identification of Bacteria from Three Different Unpasteurized Fruit Juices Sold at a Local Market in Guyana

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Authors' contributions

This work was carried out in collaboration between both authors. Authors SJ and KC designed the research. Author SJ performed the analysis and wrote the first and final draft of the research. Author KC proof-read the drafts and provided guidance in the completion of the research. Both authors read and approved the final manuscript.

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ABSTRACT

Fruit juices are widely consumed for their nutritional value. However, fruit juices that are unpasteurised may be unsafe to drink because they are not subject to heat processing and much of their human handling results in contamination. The fruits used to make these juices have resident microflora, transient bacteria from handling and equipment, and bacteria from water used to wash fruits and in some cases make the juice. The objective of this research was to determine the bacteria present in unpasteurised passionfruit, cherry, and tamarind juices respectively. Through the use of a survey, and standard methods for isolation, identification and characterisation of bacteria, the results showed that temperature and pH did not significantly affect the diversity of

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bacteria. Cherry juice had the highest number of Colony Forming Units while passion fruit juice had the lowest. In total seven (7) species of bacteria were found including *E.coli*, *S. aureus, Lactobacillus sp*, *Acetobacter sp, Bacillus subtilis, Bacillus cereus*, and *Micrococcus.* The results for the Colony Forming Units were found to be above maximum acceptable levels for fruit juices. The results of this study demonstrate that unpasteurised juices may be a threat to public health and hazardous to humans if not processed in a sanitary manner.

Keywords: Unpasteurised fruit juice; bacteriological analysis; food safety.

1. INTRODUCTION

Fruit juices can be exposed to and contaminated by bacteria as a result of poor hygienic practices, improper storage, unsanitary facilities and equipment, food handlers, exceeding the shelf-life, manure and the normal microflora of fruits [1,2,3]. The preparation of fruit juices requires human manipulation. Therefore, sanitary measures must be taken or else microorganisms will be introduced into the product [2]. Fruits must be carefully washed along with the equipment used (sterilized) and fruits with blemishes should be avoided because when the fruits are wounded or cut with a contaminated knife, via the broken external barrier, the bacteria will enter and colonize inside the fruit [4]. Unpasteurized juices are the fermentable liquid that is obtained from the fruit tissue without the application of heat. The fruits being used to make the unpasteurized fruit juice may still have certain microbes present on or inside them. Some thin skinned fruits without a hard outer covering may especially be susceptible to damage due to their soft nature [5,6]. Generally, fruits that have been wounded by insects, or fallen should not be used because of the presence of bacteria.

Fruit juices usually have low pH between 2.0 and 4.5. Most bacteria prefer a pH closer to 7. However, some bacteria can survive at a low pH, therefore making unpasteurised juices unsafe to drink [7] since some microbes can be dangerous and deadly [8,9-11]. Hence, it is of crucial importance that these manufacturers or fruit juice vendors not only satisfy the needs of the people depending on the fruit juices but also provide healthy fruit juices to them. They must be free from any bacterial contamination.

1.1 Background

Spoilage of fruit juices is indicated by a change in the appearance, smell, or taste, which is due to a proliferation of natural acid-tolerant or osmophilic micro-flora [12]. Therefore, the physicochemical parameters and organoleptic properties will deteriorate [13,14]. According to [15], pH < 4.5 acts as the principal barrier for microbial proliferation. This condition enables bacteria such as *Salmonella* and *E.coli* to survive in conditions of high temperatures, thus microbial growth is encouraged. Temperatures ranging between 0-4°C are considered safe [2].

The bacteria found in fruit juice may vary. Bacteria may be grouped according to Lactic Acid Bacteria, Acetic Acid Bacteria, Alicyclobacilli, Pathogenic Bacteria, and others. These bacterial types have different features including their ability to tolerate heat and ability to survive low pH. They may also likely be more present when certain features are present such as sour fruits, sugar content in fruits, damaged fruits, fruits with thin skins, fruits that can easily be fermented, etc.[15] According to the World Health Organisation (WHO) [16] maximum acceptable level of 100 CFU/mL for total aerobic mesophilic bacteria in fruit and vegetable juices. For coliforms, the limit is 10 CFU/mL. However, the major fruit juice bacteriological contaminants include *Staphylococcus aureus, Streptococcal spp, Klebsiella,* and *E. coli*, while other contaminants *include Salmonella typhi, Proteus spp,* and *Pseudomonas aerogenosa* [17]. In cases where tap water is used to wash fruits, different bacteria may be introduced. For example, in Guyana, a study conducted found the following bacteria in tap water: *Acinetobacter* sp., *Staphylococcus* sp., *Lactobacillus* sp., *Streptococcus* sp., *Chromobacterium* sp., *Flavobacterium* sp., *Pasteurella* sp., *Salmonella* sp., *Providencia* sp., *Micrococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. [18]. Therefore, it is possible that in cases where tap water is used to wash or prepare these juices, traces may be found. Nevertheless, consumers find the unpasteurized fruit juice, which the street vendors prepare, more appealing because of its freshness, hence tastier [17]. Consuming beverages from the roadside potentially maximizes the risk of being infected with foodborne pathogens [19]. Worldwide, situations regarding fruit juices sold in the streets remain

detrimental. For example, in India, unpasteurized fruit juice is consumed by a great portion of the population. However, microbiological testing revealed that *Salmonella* was present in the juice samples with a total coliform count of three (3) which indicated water contamination during handling [6]. Similarly in Malaysia, fruit juices that were sold in tied plastic bags or cups found that 90.3% of the overall samples were contaminated with viable bacteria [19]. In Ethiopia, 85.9% of 78 juice samples had a total viable bacterial count including a 60.3% Fecal Coliform Count with *E coli* above permissible levels [20]. It was expected that because of the usual method of preparation of these juices, there would be similarities in the bacteriological analyses conducted in the study.

1.2 Justification

In Guyana, there is no sufficient information available on the prevalence of bacterial species in unpasteurized fruit juices sold in local markets. Therefore, evaluating the fruit juices' quality is essential to protecting the health of consumers. The primary purpose of this research was to carry out a preliminary analysis of the bacteria found in commercially sold fruit juices in a local market in Georgetown, Guyana. The results of this study will guide further research on the safety of unpasteurised fruit juices, and what can be done to improve their processing.

2. MATERIALS AND METHODS

2.1 Sampling Site

The site was located in the center of the capital city (Georgetown) of Guyana. It is a place where the majority of the salespersons in Guyana go to establish their business to make a living; whether it is on a large-scale, medium-scale, or a small-scale. Hundreds of Guyanese travel there daily.

2.2 Survey

A simple visual survey was conducted to determine the common unpasteurized fruit juices sold by the local small-scale fruit juice vendors. The researchers randomly selected stalls and noted the unpasteurized fruit juices sold by the vendors, and then came to the conclusion that passion fruit juice, cherry juice, and tamarind juice were the most common juices sold among the targeted stalls. The owner of each stall was briefly interviewed on the technique or approach used to prepare the juices, where the juices were prepared and how long before they were prepared. Six (6) juice vendors were interviewed.

2.3 Sample Collection

The juice samples were collected over a three week period from the 24th of January, 2023 to the 8th of January, 2023. Two batches of juice samples were collected per week (Fig. 1).

A total of 18 samples were collected. All the samples were collected during the morning hours (before 9:00 am), stored in a cooler with ice, taken to the University of Guyana, Faculty of Natural Science, Biology Laboratory D14, and were analyzed the same day.

2.4 Preparation of Dilution Blanks and Plates

200mL of distilled water was added to a suitable beaker. A filter paper was placed on the electric balance, set to zero, and 0.18g of NaCl was measured with the help of a spatula. The 0.18g of NaCl was added to the 200mL distilled water and was stirred until the NaCl was dissolved completely. A 10ml measuring cylinder and a dropper was used to measure 9mL of saline solution, which was added to one of the test tubes that was washed. A suitable plug was formed with the cotton roll and was inserted into the test tube containing the saline solution. This step was repeated for all the other test tubes. These tubes prepared are referred to as the dilution blanks. All the dilution blanks were autoclaved. Nutrient agar plates were used as an analysis medium.

2.5 Serial Dilution

Serial Dilution Method was done and all the tubes were labeled accordingly (A1 10^{-1}, A1 10^{-2}, and A1 10^{-3}). The serial dilution procedure was repeated for the cherry and tamarind juice. The dilution tubes for the cherry juice were labeled as follows; A2 10^{-1}, A2 10^{-2}, and A2 10^{-3}, and the dilution tubes for the tamarind juice were labeled as follows; A3 10^{-1}, A3 10^{-2}, and A3 10^{-3}.

2.6 Method of Streaking and Incubation

The standard four quadrant streaking technique was used. The plates were then labeled appropriately; the researcher's name, date, medium type and sample ID. The plates were then sealed and placed into an incubator at 37°C for 48 hrs.

Fig. 1. Image showing one batch of juices that was collected

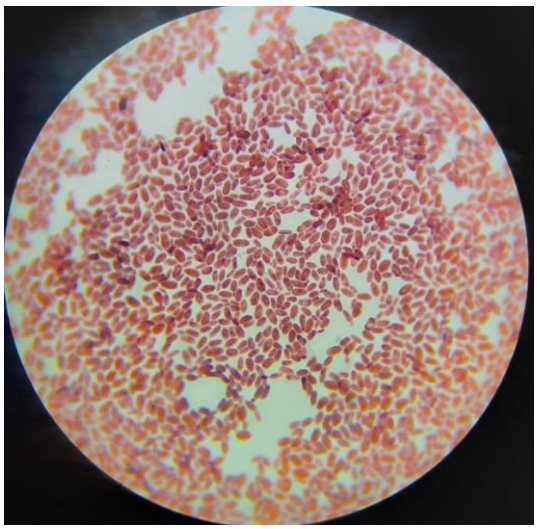

Fig. 2. Image showing Gram staining under microscope examined under x100 magnification

2.7 Colony Counting, Morphology and Identification

The Total Bacterial Count was calculated using the CFU/ml formula: Number of colonies / (Volume of culture plate * dilution factor). The above procedures were repeated for all the other unpasteurized fruit juices. Bacteria identification was done by microscopic investigation for Gram reaction. The morphological features of any suspected bacteria was determined by way of using standard method of Gram staining. [6,8].

2.8 Gram Staining

Gram Staining technique followed that of Aneja et al [15] and slides were examined under the microscope (Fig. 2).

2.9 Biochemical testing: Oxidase Testing, Catalase Testing and Coagulase Testing

Standard Tests were done for Oxidase, Catalase and Coagulase. [6].

3. RESULTS

3.1 Interview Results

Based on the results of the interview (Fig. 3), out of the six (6) fruit juice vendors, five (5) of them did not wear a mask, four (4) of them did not wear gloves, five (5) of them did not wash the equipment and bottle used respectively because they claimed that they were already washed and purchased with seals. Four (4) of them had their

workstation/kitchen outdoors, and three (3) of them prepared their juices the night before. All six (6) of the fruit juice vendors used tap water to wash the fruits.

3.2 Experimental Results

3.2.1 Total bacterial counts

The unpasteurized cherry juice showed the highest bacterial count (1.95x 10^5 CFU/ml) (Fig. 4) with an average pH of 3.6 and average temperature 23.8°C. Unpasteurized tamarind juices had the second highest bacterial count of 1.0x10^5 CFU/ml with an average pH 3 and average temperature 23.8°C. Unpasteurized passion fruit juices had the least bacterial count of 8.0x10^3 CFU/ml, with average pH of 4, and average temperature of 23.8°C. The highest pH was found to have the lowest total CFUs. The temperatures remained the same across batch collections. The number of CFUs for all juices were above permissible World Health Organisation Standards acceptable levels, thus not suitable for consumption [16].

3.2.2 Statistical Analysis - The Kruskal-Wallis Rank Sum Test (adjusted with the Bonferroni method)

There was a weak positive correlation (R = 0.11) between the CFU and temperature of the unpasteurized tamarind juice (Fig. 5).

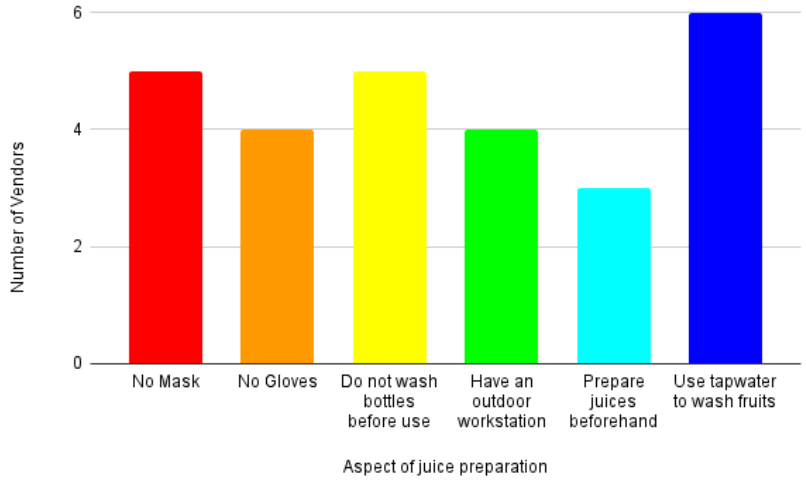

Fig. 3. Graph showing results of interview taken from six (6) fruit juice vendors

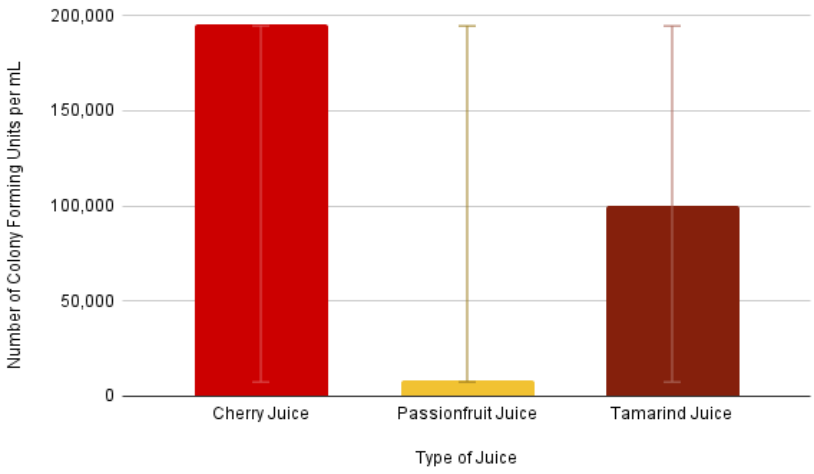

Fig. 4. Graph showing number of colony forming units per mL

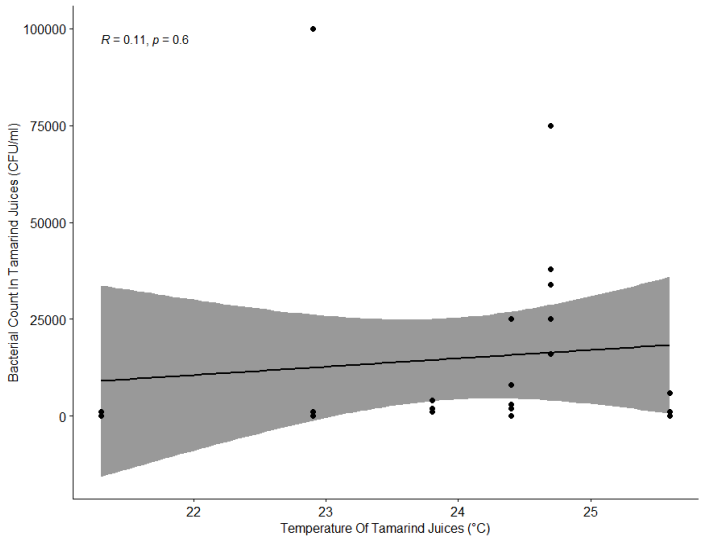

Fig. 5. Kruskal-wallis rank sum test for tamarind juice (Temperature vs CFU)

Table 1. Isolates and their morphological characteristics

Possible Isolate	Shape	Margin	Elevation	Configuration	Colour on Agar	Gram Stain
Escherichia coli	Rod	Entire	Slightly raised	Circular	Pink	Negative
Bacillus subtilis	Rod	Irregular	Flat	Circular Lobate	White	Positive
Bacillus cereus	Rod	Entire	Convex	Circular	Off-white	Positive
S. aureus	Cocci	Entire	Convex	Circular - colonial	Yellow	Positive
Micrococcus sp.	Cocci	Entire	Convex	Circular	Yellow	Positive
Acetobacter sp.	Rods	Entire	Flat	Circular	Pale	Negative
Lactobacillus sp.	Rods	Entire	Raised	Circular	White	Positive

Table 2. Biochemical characterisation of isolates

Name of possible isolate	Catalase	Oxidase	Coagulase
Escherichia coli	positive	negative	negative
Bacillus subtilis	positive	negative	negative
Bacillus cereus	positive	negative	negative
Staphylococcus aureus	positive	negative	positive
Micrococcus sp.	positive	positive	positive
Acetobacter sp.	negative	negative	negative
Lactobacillus sp.	negative	negative	positive

Table 3. Bacterial isolates and their percentage abundance

Bacterial Isolate	Cherry Juice	Passionfruit Juice	Tamarind Juice	Number of total Samples	Frequency of Isolate
Escherichia coli	6	6	6	18	100%
Bacillus subtilis	4	-	1	5	27.8%
Bacillus cereus	5	1	3	9	50%
Staphylococcus aureus	3	3	1	7	38.9%
Micrococcus sp.	4	-	-	4	22.2%
Acetobacter sp.	3	-	1	4	22.2%
Lactobacillus sp.	6	3	5	14	93.3%

The other 2 juices (passion fruit and cherry juice) demonstrated weak negative correlations (R= -0.23 and R = -0.076 respectively). Weak negative correlations were found in all the juices when comparing CFUs and pH where *R* = -0.19 (passionfruit juice), R = -0.17 (cherry juice) and R = -0.00075 (tamarind juice).

3.3 Bacteriological Analysis

3.3.1 Possible Isolates found in juices and their morphological characteristics

In the study, 18 samples were analysed for the presence of bacteria. Based on morphological characteristics, there were seven (7) isolates that were found. There were more Gram Positive bacteria than Gram Negative bacteria (Table 1).

3.3.2 Biochemical characterisation of isolates

Based on the results of the Catalase test, most of the bacteria were found to be Catalase positive. The Oxidase and Coagulase tests were mostly negative (Table 2).

3.3.3 Bacterial isolates (Percentage Abundance)

Out of 18 samples, the most frequently isolated bacteria were *E. coli*, followed by *Lactobacillus sp.* Low frequencies of isolates were found from *Bacillus subtilis*, *S. aureus*, *Micrococcus sp*. and *Acetobacter sp.*(Table 3).

4. DISCUSSION

This study presented the bacteriological analysis of unpasteurised fruit juice samples from a local market in Guyana. Overall, the juice samples tested were found to be not safe for human consumption based on bacterial loads. The pH of the samples, though low and acidic, still promoted some forms of bacterial growth, for example *E. coli* and *S. aureus* which are dangerous bacteria, responsible for food outbreaks and decreased food safety. These are pathogenic bacteria that are deadly and dangerous and can cause symptoms such as diarrhea, abdominal pain, chills, fever and nausea. [8]. Though these bacteria were found in low pH, likely due to biological processes, they regulated their internal pH and survived as pathogens [15]. It was expected that because cherry juice and passion fruit juice are usually high in sugar, that these two juices would have had lower bacterial diversity present when compared to tamarind juice. However, cherry topped the list as being the most microbially diverse fruit juice (Fig. 6).

Passionfruit had the lowest microbial diversity. Cherry has thin skin and is very prone to breakage and bruising of its skin barrier. Therefore, microbes would be able to enter easily. As a result, the high microbial diversity is not surprising [5,6]. Because cherry has a high possibility of fermenting, there was likely more acid production, hence greater presence of lactic acid bacteria (*Lactobacillus sp)* than the other two juices [16]. The high number of CFUs in the juices was likely due to the insanitary way that these juices are prepared. The preparation of fruit juices requires human manipulation; if sanitary measures are not taken then microorganisms will be introduced into the product [2]. It is also likely that bacteria was transferred into the fruit juices from the use of tap water among all of the vendors, and transient bacteria from the humans making the juice, based on the vendors' responses during the

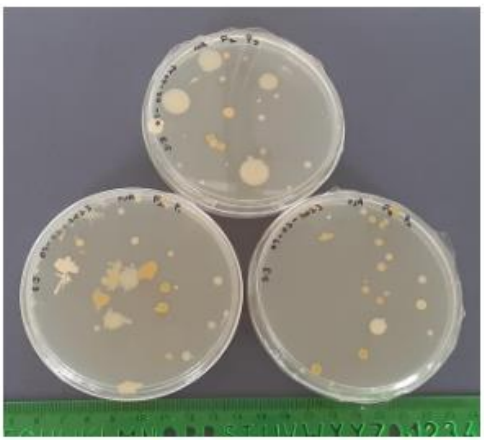

Fig. 6. Some plates from Cherry Juice samples before isolation

survey. Tap water was a likely factor in the presence of some species of bacteria. This was indicated by the presence of the two *Bacillu*s species, *Micrococcus sp.*, *Lactobacillus sp.*, and *Staphylococcus sp*., which are dominant in tap water in Guyana [18]. The presence of Staphylococci can also cause toxins [17] and lead to hospitalisation due to food poisoning. This is worrying for human consumers and poses a public health risk. The presence of *S. aureus* could mostly be as a result of food handling in an insanitary manner since *S. aureus* is unlikely to survive in low pH [15] and is present on human skin. Therefore, as indicated by the survey results, unsanitary conditions prevailed. The presence of *Acetobacter sp.* in cherry and tamarind could likely be as a result of the use of spoiled cherries and tamarinds since these fruits are not naturally rich in acetic acid [16]. Additionally, tamarind and cherries can be very sour, therefore accounting for the presence of this species. The two *Bacillus* species found were likely due to their spore producing nature and their ability to even ward off heat. *Bacillus cereus* is especially very ubiquitous so its presence is not surprising [15]. The overall results of the study were also similar to that of [6] except that *Salmonella* was not found in any of our samples. It is recommended that these unpasteurised juices are prepared in a sanitary manner and under sanitary conditions. Though this is not a perfect solution, it can eliminate the occurrence of many Coliform bacteria such as *E.coli* and *S. aureus* which can require medical attention.

5. CONCLUSION

In conclusion, seven (7) species of bacteria were found in the tested samples of passionfruit, cherry, and tamarind juices. It was revealed that cherry juice had the overall greatest number of Colony Forming Units while passion fruit had the lowest number of Colony Forming Units. The greatest microbial diversity was found in cherry juice with seven (7) species, followed by tamarind juice with six (6) species. Only four (4) species were found in passion fruit juice. The most frequent bacteria isolated was *Escherichia coli* which was likely as a result of its ability to survive even in low pH, along with its ubiquity This bacteria is threatening to human health. Further studies should be done on unpasteurised fruit juices to determine the main pathways of transmission. Consumers can also be careful when purchasing unpasteurised fruit juices noting that they may not be fit for human consumption, with the potential to cause diseases or other temporary discomforts like nausea and diarrhea.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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