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Articles

Biochemical and Bacteriological Examination of Some Selected Sliced Fruits

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Abstract

Because of their articulated traits in the preferment of the health of patrons, diets encompassing fruits are exceedingly indorsed. Throughout harvest and/or post-harvest, fruits come in interaction with water, soil, dust, and so many other forms of management. This eventually exposes these fruits to a lot of microorganisms, some of which can be pathogenetic. The current study analysed the bacteriological content of some selected sliced fruits (pineapple, Pawpaw and watermelon) sold in Azare, Bauchi State, Nigeria. The samples were subjected to a variety of physiochemical, microbial and biochemical tests which include determination of temperature and pH, gram staining, catalase, coagulase, indole, and urease tests. Results showed the percentage frequency of the isolated bacteria to be; 26.5 %, 35.2 %, 14.7 %, 23.5 %, for E. *coli, Pseudomonas* spp, *Staphylococcus* spp, and *Bacillus* spp respectively. The most occurring bacterium observed in this study was *Pseudomonas* spp with the occurrence rate of 35.2 % while *Staphylococcus spp and Bacillus spp* had the least occurrence rates of 5.0 % and 8.0 % respectively.

Keywords: physiochemical, bacteriological, catalase, coagulase, indole, urease.

1. Introduction

Because of their expressed attributes in promotion of the health of consumers, diets containing fruits are highly recommended (Igiehon et al., 2020). Fruits contain a significantly high concentrations of minerals, vitamins, phytochemicals and lots of electrolytes (Amao, 2018).

Consumption of sufficient amounts of fruits lowers blood cholesterol levels, controls blood pressure, reduces the risk of some heart diseases, and prevents some kinds of cancer (Wang et al., 2014).

During harvest and/or post-harvest, fruits come in contact with water, soil, dust, and so many other forms of handling (Ramaswamy, 2015). This ultimately expose these fruits to a lot of microorganisms, some of which can be pathogenetic (Castro-Ibáñez et al., 2017). A lot of fruits are commonly sold as cut or sliced fruits to entice the consumers. These vended or ready-to-eat fruits, may include watermelons, pineapple and pawpaw and, cucumbers, mangoes, oranges, etc. They are usually displayed at strategic places or carried around by hawkers to be sold to buyers for

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immediate consumption without necessarily rinsing or washing them because they have already been prepared and packaged in small polythene bags (Alegbeleye et al., 2018). Because of their accessibility, convenience, and relatively cheaper prices than whole fruits, these ready to eat or vended fruits have become ubiquitous (Vidhya Ganesh, 2013).

Consequently, they have progressively developed into staples owing to the current modernization, industrialization, economic downturn, materialism, and absence of abundant time to prepare a suitable meal in most homes (Satterthwaite et al., 2010). This trend has been seen to signal a great risk to consumer health because it is difficult to ascertain the hygienic processes the fruits are subjected to after harvesting, during processing, and before packaging (Alegbeleye et al., 2018). The processing is usually done lacking appropriate stowing environments, thereby exposing these fruits to heat, flies, cockroaches, rodents, dust, pathogens, dirt, and other environmental pollutants (DuFour, 1995; Alegbeleye et al., 2018).

Moreover, these fruits are vended by uninformed retailers or local hawkers who have petite or no familiarity with food hygiene, nutrition, and pathogens (García, Heredia, 2017).

Usually, non-pathogenic microflora is the common flora found in fruits, the epidermal layer resists the penetration of microorganisms (Eisenstein, 2020). These protection by the epidermal layer is removed by cutting and slicing, hence microbes could easily invade the internal tissues (Ramaswamy, 2015), consequently, snowballing the risk of food intoxication caused by pathogens such as fungi, parasites, viruses, and bacteria in consumers (Hailu et al., 2014).

To eradicate this delinquent, numerous valuations have been carried out to study the microbial contaminants of some of these vended fruits. Nonetheless, it is essential to further appraise and reconnoiter the microbial qualities of some of these vended fruits in order to fashion consciousness of the peril accompanying their consumption. To a great extent, this will assist to lower the outbreak of diseases, severe health crises, and death.

2. Materials and methods

Study area

The study area is in Bauchi State, Nigeria. The city is located between latitudes (9° 3'and 12° 3'N and longitudes 8° 50' and 11° E) which has covered the total area of 49,119 km² (18,965 square meter) representing about 5.3 % of Nigeria's total land mass.

Sample Collection

A total of 15 samples consisting 5 each of pineapple, Pawpaw (Carica papaya) and Watermelon were randomly bought from different selling outlets in Wuntin Dada market Bauchi with sterile gloves. Each sample was kept in a sterile low density polythene bag and labelled. All samples were transported to the laboratory in special boxes for analysis within 1-2 hours of collection.

Determination of Temperature and pH

The temperature of the samples was determined using a liquid-in-glass thermometer. 10 ml of the sample was dispensed into a beaker. The pH was determined with the standardized pH meter.

Bacteriological Analysis of the Samples

The total bacterial count was determined for each of the fifteen (15) sliced samples of watermelon, pineapple and pawpaw that were collected randomly from five (5) different vendors within Wuntin Dada Markets using microscopic examinations and standard biochemical tests.

Preparation of Media:

The media were in commercial dehydrated forms and were prepared according to the manufacturer's instructions.

Isolation and Enumeration of Bacteria from Samples:

A sterile knife was aseptically used to cut a portion from each of the watermelon, pineapple and pawpaw samples, after which 100 grams of each sample was weighed using a weighing, balance and was transferred into a blender. 110 ml of sterile distilled water was added and aseptically blended at the speed of 15,000 to 20,000/rpm for 3 minutes. The homogenates were further mixed by shaking and 1.0 ml was pipetted into a test tube containing 9.0 ml of distilled water and was diluted serially to obtain the desired appropriate dilutions (10^1 to 10^5). Then, 1.0 ml of each dilution of the homogenates was pipetted and introduced into each of the correspondingly labelled petri dishes in duplicates.

5 ml of nutrient agar was poured into each petri dish within 15 minutes of the time of original dilution. The sample dilution and the nutrient agar medium was mixed thoroughly by swirling and allowed to solidify.

The inoculated plates were invertedly incubated at 37±0.5°C for 24-48 hours after which all plates with 30-300 colonies were counted, recorded and expressed as colony forming units per mile (cfu/ml) of the sliced watermelon, pineapple and pawpaw samples analyzed. Discrete colonies were streaked onto fresh agar to obtain pure cultures of the different isolates. Isolates were maintained on nutrient agar slants and stored at 4°C for further tests. These procedures were repeated on five different occasions for each of the samples and the average values recorded.

Identification of Isolates

Following repeated sub-culturing, pure cultures of the different isolates were obtained, characterized and identified using biochemical tests.

Gram staining

A smear was prepared on a clean, grease-free glass microscope slide and allowed to air dry. The slide was then flooded with crystal violet for one minute, rinsed with distilled water. Lugol's iodine was then poured on it and also left for 30 seconds after which the slide was rinsed with distilled water. Acetone was used to decolorize and was washed immediately and counterstained with safranin 0 for 30 seconds. The stain was washed off with distilled water and allowed to air dry. The stained smear was examined under oil immersion objective 100 X.

Catalase test

Two drops of 3 % hydrogen peroxide were placed on a clean, grease-free microscope glass slide after which a loopful of the organism colony was added. A positive test was indicated by evolution of bubbles while in a negative test no bubbling and frothing was seen.

Coagulase test

A smear of the culture colony was mixed with human plasma with a sterile wire loop. The slide was held up and rocked back and forth for one minute. Clumping of cells were apparent in the bacterial suspension mixed with plasma for coagulase positive.

Indole test

The organism was grown in 5 ml of peptone water after 24 hours of incubation, Kovacs iodine reagent was added and shaken gently. A positive reaction was indicated by the development a red colour in the reagent layer above the broth within one minute, while the yellow colour was retained for negative reaction.

Urease test

Urease medium was inoculated with test bacteria colony and incubated at 37°C for 24 hours. The development of a bright pink or red colour indicated a positive reaction.

Motility test

The stabbing technique was used with test tubes containing sterile semi-solid nutrient agar. A sterile needle was used to pick up each isolate colony from their pure cultures. Each test tube was stabbed to a depth 1-2 cm short of the bottom of the tubes with the needle that had been rubbed with each isolate. The line of inoculation was not sharply defined and the rest of the medium was somewhat cloudy for motile organisms, while growth was restricted to the line of inoculation and it became sharply defined for the non-motile organisms.

3. Results

Table 1. Frequency of Occurrence of Bacterial Isolates from the Sliced Pineapple watermelon and Pawpaw Samples

Bacterial Isolated	Frequency of Occurrence	% of Occurrence
E. coli	9	26.5
Pseudomonas spp	12	35.2
Staphylococcus spp	5	14.7
Bacilluspp	8	23.5
Total	34	100 %

Table 2. Biochemical and Morphological Characterization of Recovered Bacterial Isolate

Gram reaction	Indole	CGL	CAT	MOT	URS	Isolate
-ve rods in pairs	+	+	+	+	-	*E. coli*
-ve rods in chain	-	-	+	+	-	*Pseudomonas spp.*
+ve long rods	+	-	+	+	-	*Bacillus spp.*
-ve cocci in cluster	+	+	+	-	-	*Staphylococcus spp.*

Key: CGL = Coagulase; CAT = Catalase; URS =Urease; MOT = Motility; Test; +ve = Positive; -ve = Negative.

4. Discussion

The relatively high bacterial count detected in this study might be attributed to environmental factors such which includes exposure of fruits (sliced pineapple pawpaw or watermelon) to air, dust, type of water used in processing, and personal hygiene of the handlers, similar reasons were identified by García and Heredia (2017) and Castro-Ibáñez et al. (2018). The presence of these organisms can be linked to a number of factors; such as improper handling and processing, use of contaminated water during washing, cross-contamination from the fruits and vegetables or the use of dirty processing utensils like knives and trays, other studies agree completely with these findings of Alegbeleye et al, (2018) and those of Eisenstein (2020). The frequency of isolation of *Staphylococcus* spp. may be explained by the fact that human beings, i.e. processors or vendors, carry these organisms on/in several parts of their bodies and also in the nasal passages and on the skin surfaces as also swotted by Igiehon (2020). This organism can be introduced into the fresh sliced Pineapple, Pawpaw and Watermelon during handling, processing or vending and may lead to staphylococcal food poisoning, gastroenteritis.

The high level of *Escherichia coli* indicated a feacal contamination and water pollution respectively which implicated the processing and rinsing water as possible sources of contamination. This might cause bloody diarrhea and gastroenteritis in individuals who consumes such contaminated pineapple.

The low level of isolation of *Staphylococcus spp* is surprising. Generally, *Staphylococcus* spp. is salt-loving bacteria as expressed by Wang et al. (2014).

The presence of *Bacillus* spp. agrees with the report of Satterthwaite et al. (2010) that Bacillus spp. is the major spoilage organism in juices and causes emetic syndromes (which is characterized by acute-onset nausea and vomiting), and diarrheal syndromes.

5. Conclusion

It obvious that sliced Pineapple, Pawpaw and Watermelon are one of the most popular fruits that people rush to purchase not only for its low price but for it nutritional benefits. Microorganism encountered in this study were as a result of contamination from one source or the other which include; air, improper handling, unclean water and utensils used in the processing and personal hygiene.

The bacteria isolated are of public health importance which may cause diseases such as gastroenteritis, bacteremia.

The above findings therefore demonstrated the need for adequate evaluation of the physiochemical (including pH) characteristics of the Pineapple Pawpaw and Watermelon being sold to the populace in order to ensure safety and healthy eating.

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