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MICROBIAL EVALUATION OF FRESH-CUT WATER MELON FROM THREE LOCATIONS IN AUCHI, NIGERIA

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ABSTRACT

Water melon has become one of the most consumed fruits due to its nutritional and health benefits. As a result of its high cost consumers now purchase the sliced polythene bag packaged water melon. In this study bacteria and fungi associated with sliced water melon sold at three locations in Auchi were evaluated. Three samples were purchased from the three locations and analyzed microbiologically using standard procedures. Samples for bacterial examination were cultured on Nutrient agar and MacConkey Agar while samples for fungal examination were cultured on Potato Dextrose Agar. The colony forming units (CFU/g) of the bacterial isolates ranged from 4.4 x10^5 to 9.0 x 10^5 while the fungal isolates ranged from 1.0 x10^{5}to 4.0 x10^5. The suspected bacterial isolates were identified as Escherichia coli, Klebsiella spp and Staphylococcus aureus while the fungal isolates were identified as Mucor spp, Saccharomyces spp and Rhizopus spp. The incidence of Escherichia coli encountered in this study pose a threat to the consumer's health as an enteric bacterium. To prevent foodborne diseases and foodborne poisoning there is need to educate the sellers on high personal hygiene and general good practices when slicing and packaging fresh-cut water melon.

Keywords: Bacterial, Foodborne, Fresh-cut, Fungal, Water melon

INTRODUCTION

Watermelon (*Citrullus lanatus*) belong to the family of *Cucurbitaceae* (Tseng *et al*., 2022).Water melon is a staple summer fruit, it is mostly consumed as fresh-cut fruit or juice (Perkins-Veazie *et al*., 2013) or as a dessert (Paris, 2020). Watermelon is a natural source of antioxidants, Vitamin C and lycopene (Naz *et al*., 2014). Lycopene present in watermelon helps to improve human health (Asante *et al*., 2020). Lycopene is also known to control chronic diseases such as diabetes, cardiovascular diseases, and some forms of cancer (Figueroa *et al*., 2011; Maoto *et al*., 2019). Fresh-cut water melon is among the fresh-cut fruits now being widely consumed in Nigeria as a result of its convenience and affordability. Watermelon grows on the ground; this makes it susceptible to contamination through its contact with soil and irrigation water pathogens (Tseng *et al*., 2022). Fresh-cut fruits are vulnerable to foodborne pathogens; among which are *Salmonella* spp, Shiga toxin-producing

Escherichia coli, *Campylobacter* spp, *Staphylococcus aureus* and *Listeria monocytogenes* (CDC, 2022). *Saccharomyces cerevisiae*, *Rhizopus stolonifer* and *Mucor* spp were fungi associated with sliced water melon contamination (Nwachukwu *et al*., 2008). Water melon is prone to foodborne pathogens contamination due to its rough peel that makes it easy for bacterial attachment and survival. Water melon near neutral pH (6.67), high water content (0.97-0.99) and high sugar content also increase its vulnerability to microbial activities (Tseng *et al*., 2022). Foodborne pathogens may also invade the interior surfaces of fresh-cut water melon during peeling, slicing, trimming and other process like packaging, handily and marketing (Barro *et al*., 2007). The aim of this study was to determine the microbiological load (bacteria and fungi) of fresh-cut water melon sold by street vendors.

MATERIALS AND METHODS

Sample procurement: Fresh-cut water melon fruits were randomly purchased from three vendors from three locations in Auchi metropolis (Sample A- Jattu, Sample B- Sabo and Sample C-Uchi market).

Methods: The samples were transported in a sterile container to the laboratory and analyzed within 1 - 2 h after collection at Microbiology Laboratory of Food Technology Department, Auchi Polytechnic, Auchi, Edo State, Nigeria. MacConkey agar (Antec Diagnostics Products, UK), Nutrient agar and Potato Dextrose agar (International Diagnostics Group, UK) were prepared and used for the isolation and enumeration of bacteria and fungi. The various media were prepared, as specified in the manufacturer's manuals (Asante *et al*., 2020).

Isolation and enumeration of bacteria and fungi

One gram (1.0 g) from each watermelon sample was obtained and homogenized using a sterile mortar and pestle. The homogenate was added to 9.0 ml sterile peptone water in a test tube and diluted serially to 10^4. From the 10^4 dilution, 0.1 ml was plated onto the different media in duplicates. The MacConkey agar and Nutrient agar plates were incubated at 37^{o}C for 24 h to obtain the total viable bacterial counts, while the Potato Dextrose agar plates were incubated at 28^{o}C for 72 h to obtain the fungal counts. Discrete colonies were streaked onto fresh agar to obtain pure cultures.

Identification of Isolates

Bacterial isolates were identified by carrying out Gram staining and other biochemical tests was carried out based on the method of Cheesbrough (2006). The biochemical tests performed here include catalase, oxidase, indole, coagulase, citrate and Sugar fermentation test.

Gram Staining

A thin smear of the isolates was carried out on different slides with the aid of a wire loop and left to dry and after they will be heat fixed and allowed to cool. Then the different smears were covered with crystal violet stain for 30-60seconds and rapidly washed off with clean water. Then the smears were covered with Lugol's iodine for 30-

60seconds and rapidly washed off with clean water. The smears were decolourised rapidly with alcohol and washed out immediately with clean water. Then the smears were covered with safaranine for 30-60seconds and washed immediately with clean water. The stained smears were then allowed to air-dry. After drying, a few drops of oil immersion was dropped on the stained smears and viewed with the aid of a microscope (×100 oil objective lens) to check for the microscopic properties of the organisms like the Gram reaction, morphology (Cheesbrough, 2006).

Biochemical Tests

Catalase Test

The discrete colonies of each of the isolates were collected with a glass rod and emulsified in a drop of hydrogen perioxide (H_2O_2). Bubbles of gas indicated a positive result according to Cheesbrough, (2006).

Motility test

Motility test

Freshly prepared bacterial isolates of 24h were inoculated into motility medium (peptone water 0.1g, agar-agar 0.05g, NaCl 0.05g, distilled water 1L). The motility medium was sterilized at 121^0C for 15min by autoclaving. The cooled sterilized motility medium was stabbed inoculated at the centre of the medium and incubated at 37^0C for 48h anaerobically (Cheesbrough, 2006).

Indole Test

Each of the bacterial isolates was inoculated into 5ml of sterilized prepared peptone

water which was contained in different test tubes using a wire loop. Test tubes containing the organisms were incubated at 37°C for 48hours. After incubation period, 3-4drops of indole reagent known as Kovac's reagent was added and shaken gently. A positive result gave a red surface layer after 10minutes while a negative result gave a no red surface layer after 10minutes according to Cheesbrough, (2006).

Oxidase Test

A piece of filter paper was placed in a clean petri dish and 2-3drops of freshly prepared oxidase reagent was added. With the aid of a wooden stick, discrete colonies of the isolates was collected separately and smeared on

the filter paper. A positive result gave a purple-blue colouration after10seconds while a negative result gave no such colour after 10seconds according to Cheesbrough, (2006).

Coagulase Test

A drop of distilled water was placed on each end of a slide and a colony of the test organism was emulsified in each of the drops to form a thick suspension. A loopful of plasma was added to one of the suspensions and swirled gently. A positive result showed clumping after 10secconds while a negative result showed no clumping after 10seconds according to Cheesbrough, (2006).

Citrate Utilization Test

The Simon's citrate agar was prepared according to specification of the manufacturer in sterilized Petri dish; it was inoculated with the test organism and incubated at 37˚C in the incubator for 3days. A change in colour from green to blue indicated a positive result and a negative result remained green.

Sugar Fermentation Test

The fermentation medium (Peptone water 2.0g, Sodium chloride 2.0g, Dipotassium hydrogen phosphate 3.0g, Methyl red (indicator) 1.0g, Distilled water 1L) was prepared and sterilized with the indicator and Durham's tube had no air bubbles in them. The sugar solution was autoclaved at 10 ibs/sq inch pressure for 10minutes and 0.5ml of the sugar was added to sterile peptone water. The fermentation tubes were inoculated with the test organism. Negative control was maintained for all the sugar. The tubes were incubated at 37 0C for 24-48hrs. Colour change was observed.

Fungal identification

Fungal identification and classification were based on their macroscopic and microscopic features. The macroscopic features were based on the shape, colour and physical appearance of the colonies, while the microscopic characterization was carried out using lactophenol cotton blue and examined using x40 objective.

Statistical Analysis

The microbial counts were subjected to Analysis of Variance (ANOVA) using the post Hoc Tests. The analyses that were used were Descriptive measures and repeated measures at the probability level of $p = 0.05$ using IBM SPSS software (version 23.0).

Results and Discussion

Bacterial and fungal loads of the fresh-cut water melon were determined as represented in Table 1 below. There was no significant difference ($p \geq 0.05$) for each water melon sample. Bacterial isolates colony forming units

(cfu/g) ranged from 4.4 x10^5 to 9.0 x 10^5 while the fungal isolate colony forming units ranged from 1.0 x10^5 to 4.0 x 10^5.

Table 1: Bacterial and Fungal colony forming units (cfu/g) from fresh-cut water melon samples

SAMPLES	BACTERIAL ISOLATES	FUNGAL ISOLATES
A	9.0 X 10^5 ±1.05^a	1.0 X 10^5 ±1.05^b
B	7.0 X 10^5 ±1.10^b	3.0 X 10^5 ±1.05^a
C	4.4 X 10^5 ±1.05^c	4.0 X 10^5 ±1.05^a

Keyword: Means within column with different superscript are significantly different P≥0.05. N=3±SD

Fresh-cut fruits can easily be contaminated with bacteria and fungi from vendors through mishandling (Asante *et al*., 2019). The risk of foodborne illness increases through the consumption of fresh-cut fruit since the vendors do not have knowledge of personal hygiene (Asante *et al*., 2020). Bacterial load of sample C is within the permissive range (5.2 log CFU/ml) recommended for fruits (FAO, 2005). Samples A and B were unwholesome for consumption since they exceeded the maximum permitted range. The result of the bacterial load in this study is within the range reported by Ocans De Jesus *et* al. (2022), who reported the microbiological quality and presence of enteropathogenic bacteria in orange juice with bacterial load ranged of 1.80- 5.47CFU/ml. This high load of bacterial contamination is similar to the study carried out by Iqbal *et* al. (2015) who reported the incidence of microbial contamination in unpasteurized fruit juice. Nawawee *et* al. (2019) reported high microbial load in milk and fruits while Ferrari *et al*. (2021) reported unsafe microbial load in foods sold on the street. Afreen *et* al. (2019) also reported high microbial loads in fruit juice. The occurrence of high load of bacterial in this study showed that fresh fruits can serve as reservoir for foodborne illnesses. The incidence of *Escherichia coli* among the bacterial incriminated showed lack of sanitary hygiene by the food vendor since *Escherichia coli* is an enteric microorganism. Fruits are rich in carbohydrate that supports the survival of these pathogenic bacteria (Vantarakis *et* al., 2011). These bacteria have been reported to cause ill health to humans as low as 10-100 cells (Kaczmarek *et al*., 2019). Fungal load ranged in this study was 1.0 x10^5 - 4.0 x10^5 which were within the ranged (2.48- 2.86 x 10^5 reported by Oranusi and Olorunfemi. (2011) but contrary to 5.28-5.75 $\log_{10}$ CFU/g reported by Nwachukwu and Osuocha. (2014).

The Morphological, microscopic and biochemical characteristics of the bacterial isolates were represented in Table 2 below.

Table 2: Morphological, microscopic and biochemical characteristics of bacterial isolates from fresh-cut water melon samples

CHARACTERISTICS	ISOLATE 1	1SOLATE 2	ISOLATE 3
Morphology			
Colour	Pink	Pink	Yellow
Shape	Regular	Large	Regular
Microscopic			
Cell type	Rod	Rod	Cocci
Cell arrangement	Single	Single	Cluster
Gram's reaction	Negative	Negative	Positive
Motility	Positive	Negative	Negative
Biochemical Tests			

Catalase	Positive	Positive	Positive
Coagulase	Negative	Negative	Negative
Indole	Positive	Negative	Negative
Oxidase	Negative	Negative	Negative
Citrate	Negative	Positive	Negative
Sugar fermentation Tests			
Glucose	Positive	Positive	Positive
Lactose	Positive	Positive	Positive
Maltose	Negative	Positive	Positive
Mannitol	Positive	Positive	Positive
Raffinose	Negative	Positive	Positive
Sorbitol	Positive	Positive	Positive
Ribose	Negative	Negative	Positive
Sucrose	Positive	Positive	Positive
Probable Organisms	*Escherichia coli*	*Klebsiella* spp	*Staphylococcus* spp

The incidence of *Staphylococcus* spp as one of the contaminating bacteria in the fresh-cut water melon in this study is an indication of contamination from the nasal cavity which could be as a result of sneezing and coughing or from the vendor's skin since *Staphylococcus epidermis* is a normal flora of the skin.

Cultural and microscopic characteristics of the fungal isolates of the fresh-cut water melon are represented in Table 3 below.

Table 3: Cultural and microscopic characteristics of the fungal isolates of the fresh-cut water melon

Isolates from water melon samples/Probable organisms	Cultural Characteristics	Microscopic characteristics
A, B, C (*Mucor* spp)	White and fluffy this later turned grey. Reverse side is white	Sparsely septate, broad hyphae. Sporangiospore were visualized.
A, B, C (*Saccharomyces* spp)	Raised, sooth, moist, glistering Creamy colonies	
B, C (*Rhizopus* spp)	White and fluffy this later turned dark as it aged.	Non-septate,spherical columela which borne spores with hyphae present in the rhizoid

Fungal isolated from the fresh-cut watermelon in this study were *Mucor* spp, *Saccharomyces* spp and *Rhizopus* spp. *Mucor* and *Rhizopus* spp results in spoilage while *Saccharomyces* spp bring about fermentation. *Rhizopus* and *Mucor* spp are air-borne filamentous fungi; therefore there occurrence in the watermelon could be from atmospheric air. Similar fungi were reported by Nwachukwu *et al*. (2008), who reported studies on microbiology of polythene-packaged sliced watermelon. In contrast, Abdulkareem and Odeh. (2021) only isolated *Mucor* spp from sliced watermelon. The sources of the microbial load associated with the fresh-cut water melon in this study

could be from the use of contaminated water to rinse the water melon before cutting, lack of personal hygiene of the vendors and the environment where the water melon was been sold.

Conclusion

The fresh cut watermelon could be potential sources of the spread of foodborne illnesses. The presence of these microorganisms can be associated with poor agricultural practices, unhygienic processing of the fresh cut fruits and the use of poor-quality water. To prevent this high incidence of microbial load in fresh cut fruits, there is need to enlighten fruit vendors on proper personal hygienic. Clean, microbial free water should be used to rinse the watermelon before cutting. Polythene bags used for packaging should be sealed with sealing machine asceptically.

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