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MICROBIOLOGICAL ASSESSMENT OF WATERMELON FROM MARKETS IN BENIN CITY, NIGERIA

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ABSTRACT

In this study, samples of watermelon (Citrullus lanatus) were obtained from different locations (Oba market, Uselu market and New Benin Market) in Benin City, Edo State Nigeria, to examine their microbiological deterioration when stored at room (22℃) and refrigerator temperature (4℃) for 5 days. Isolation and identification of the microbial species present in the samples were performed using cultural, morphological and biochemical characteristics. The bacterial counts ranged from 2.7×10^4 CFU/ml to 6.87×10^4 CFU/ml and 1.23×10^4 CFU/ml to 2.87×10^4 CFU/ml, at 22℃ and 4℃ respectively. On the other hand the fungal counts ranged from 1.17×10^4 CFU/ml to 2.77×10^4 CFU/ml and 1.93×10^4 CFU/ml to 4.00×10^4 CFU/ml at 4℃ and 22℃ respectively. The microbial load was significantly reduced ($P < 0.001$) at refrigerator temperature compared with sample storage at room temperature. The microbial isolates identified were Enterobacter aerogenes, Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus fermenti, Staphylococcus aureus, Aspergillus niger, Saccharomyces cerevisiae, Fusarium oxysporum, Rhizopus stolonifer, and Mucor sp. B. subtilis, P. aeruginosa, S. aureus and Mucor sp. were the most frequently isolated microbes at room temperature, while P. aeruginosa, B. subtilis and F. oxysporum had the highest frequency of occurrence at refrigerator temperature. The results obtained in this work suggest that proper storage temperature and sanitation could significantly influence the shelf-life and microbial diversity of freshly cut watermelon.

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1. INTRODUCTION

Watermelon (*Citrullus lanatus*; family: *Cucurbitaceae*), also known as the sweet desert watermelon, is believed to have originated in northeastern Africa over 5,000 years ago and is

cultivated for water and food (Paris, 2015). Watermelon consist of approximately 93% water, hence the name (Erhirhie and Ekene, 2014). *C. lanatus* is highly nutritional, especially the rind and seed. Several studies have outlined the numerous biological and pharmacological benefits of watermelons (Ahn et al., 2011; Bhardwaj et al., 2012; Madhavi et al., 2012; Olamide et al., 2011; Oluwole et al., 2013). Some bacterial and fungal species were sensitive to ethanolic, chloroform and hexane extracts from the fruit in comparison with popular antimicrobial drugs (Hassan et al., 2011). Also, the methanolic extracts are a potential anti-inflammatory mediator with promise as a food medicine (Gill et al., 2010).

Microorganisms are ubiquitous in air, soil and water. Therefore, there is a large diversity of the microbiome on the surfaces of animals and plants. Watermelon is a suitable host for the colonization of microorganisms, and these organisms involved could be spoilage or pathogenic. Because of the high moisture content in watermelon, spoilage bacteria easily invade the fruit, especially in poor storage conditions modifying the texture, flavor, color and/or appearance (Ogunbanwo et al., 2013). Opportunistic pathogens arise during pre- and post-harvest or by cross-contamination when handling or processing the fruits (International Commission on Microbiological Specifications for Foods ICMSF, 1996). They may result in infections such as gastroenteritis. Hence, public health professionals place emphasis on the quality and hygiene of fruits (Walsh et al., 2014).

In Nigeria, most watermelons are grown and harvested in the northern parts and are distributed to all parts of the country for consumption (Titilayo and Salome, 2014). The farmers, vendors and final consumers all play vital roles in preserving the overall organoleptic properties of the fruits, including reducing contamination and invasion by pathogenic and spoilage organisms. Whole watermelons can be expensive, so roadside vendors frequently prepare the pre-cut alternative. Studies conducted on pre-cut watermelon in the northern (Kano), eastern (Imo and Abia), and western (Oyo and Ogun) parts of Nigeria revealed a high level of either pathogenic or spoilage organisms such as *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Lactobacillus* spp., which are a reflection of the sanitary conditions and habits involved with handling the fruits (Chukwu et al., 2010; Izah et al., 2015; Oranusi and Olorunfemi, 2011; Titilayo and Salome, 2014). However, little information is available on the microbiome of the whole watermelon and the effect of different storage conditions. Hence, the aim of this study was to assess the microorganisms associated with watermelons purchased from markets in Benin City at room (22°C) and refrigerator (4°C) storage temperatures over a specified time period.

2. MATERIALS AND METHODS

2.1. Sample Collection

The study was carried out in Benin City, Edo State, Nigeria. One whole watermelon was purchased from local vendors at Oba, New Benin and Uselu Markets. The samples were placed in a sterile polyethylene bag and transported immediately to the laboratory for analysis.

2.2. Media Preparation

Nutrient agar (NA, containing beef extract 3 g, Agar No.2 12 g, Peptone 5 g, sodium chloride 8 g and distilled water 100 ml) and potato dextrose agar (PDA, containing potato infusion 4 g, D (+) glucose 20 g and agar-agar 15 g) were prepared according to the manufacturer's instructions (Difco). NA and PDA were used in the isolation and enumeration of bacteria and fungi respectively.

2.3. Experimental Design and Setup

Each watermelon sample purchased from the three locations was cut into three parts. One part was used for initial analysis (day 0) and the other two parts were subjected to room (22°C) and refrigerator (4°C) temperature storage respectively for 5 days. Each sample was analyzed daily to isolate and characterize contaminating bacteria and fungi.

2.4. Isolation and Enumeration of Bacteria and Fungi

Ten grams of the sample were homogenized in a sterile mortar and pestle in 90 ml distilled water. One milliliter of the homogenized sample was added to 9 ml of distilled water and serially diluted. Following serial dilutions, 0.1 ml of the 10^{-2} dilution was plated aseptically on NA and PDA media using the pour plate method (Cheesbrough, 2006). The NA plates were incubated at 37°C for 24 h to obtain the total viable bacterial counts and the PDA plates were incubated at 28 ± 2°C for 72 h to obtain fungal counts. Resulting colony counts of the isolates were determined in colony forming units per ml of inoculum (CFU/ml). To obtain pure culture of isolates, discrete colonies were streaked onto fresh media.

2.5. Identification of Bacterial and Fungal Isolates

Pure cultures of bacterial isolates were maintained in NA slants and stored at 4°C for confirmative tests. Biochemical tests such as Gram-staining, catalase, indole, urease, oxidase, citrate utilization, coagulase testing and sugar fermentation tests were used in distinguishing the different bacterial isolates (Singh, 2009). Fungal identification was based on macroscopic and microscopic features of colonies as previously described (Cheesbrough, 2006). The macroscopic features were the shape, color and physical appearance of colonies, while microscopic examination constituted spore staining and subsequent viewing under a microscope. Spore staining was carried out using a lactophenol cotton blue green reagent on a heat-fixed smear slide for 1 min. After the addition of 95% ethanol for 30 s, the slide was rinsed and viewed under a light microscope with a 40× objective lens.

2.6. Statistical Analysis

Statistical analysis was carried out using central tendency and dispersion as descriptive statistics. A paired *t*-test was used to determine significant differences in microbial counts at the different storage temperatures. Single factor analysis of variance (ANOVA) was used to test for significant differences in microbial counts in samples from the different sites

(markets). Where significant difference was recorded, the Duncan multiple range test was used to determine the source of the significant difference. All statistical analysis was carried out using the SPSS 16.0 statistical program (Ogbeibu, 2005).

3. RESULTS AND DISCUSSION

Table 1 shows the total bacterial and fungal counts at room (22°C) and refrigerator (4°C) temperature for 5 days of storage. For the analysis at refrigerator temperature, microbial counts were not recorded at day 0 because the samples needed to be stored for at least 24h before making such measurements. However, from day 1, the bacterial counts ranged from 2.7×10^4 CFU/ml to 6.87×10^4 CFU/ml at 22°C and it ranged from 1.23×10^4 CFU/ml to 2.87×10^4 CFU/ml at 4°C, while fungal counts ranged from 1.17×10^4 CFU/ml to 2.77×10^4 CFU/ml at 4°C and it ranged from 1.93×10^4 CFU/ml to 4.0×10^4 CFU/ml at 22°C (Table 1).

Table 1: Mean bacterial and fungal counts for 5 days in different storage conditions

	Bacterial count (CFU/ml)×10^4 *P-value < 0.001		Fungal count (CFU/ml)×10^4 *P-value < 0.001	
**Day	Room temperature (22°C)	Refrigerator temperature (4°C)	Room temperature (22°C)	Refrigerator temperature (4°C)
0	0.76		0.83	
1	2.70	1.23	1.93	1.17
2	3.97	1.73	2.40	1.70
3	6.27	2.27	3.07	1.93
4	6.87	2.63	3.63	2.33
5	6.37	2.87	4.00	2.77

* The P-values compare the counts for both temperatures. For all days and counts, P < 0.001 for 4°C compared with 22°C. **Day 0 indicates the initial analysis of samples.

Even though there was an increase in the microbial counts at both temperatures for 5 days, these counts showed statistical significant differences between temperatures ($P < 0.001$). Ogunbanwo et al. (2013) reported a similar observation as they observed an increase in the microbial counts in refrigerated watermelon juice stored for 15 days.

Initial analysis (i.e. day 0) of pre-cut watermelon from previous studies in Nigeria showed varying microbial counts that differed from our analysis. Ready-to-eat street-vended watermelon had microbial counts ranging from 9.6×10^5 CFU/g to 2.0×10^6 CFU/g in Umuahia (Nwachukwu and Osuocha, 2014). Sliced and packed watermelon samples purchased from 12 vendors in Oyo State had microbial counts ranging from 0.8×10^4 CFU/g to 6×10^4 CFU/g (Titilayo and Salome, 2014). Microbial counts of street-vended watermelons from Ota ranged from 3.0×10^6 CFU/g to 8.2×10^6 CFU/g (Oranusi and Olorunfemi, 2011). This variability can be dependent on the location, hygiene and sanitary quality adopted in the preparation of the watermelon sold. The International Commission on Microbiological Specification for Food has placed a standard on the acceptable limit ($0 - 10^3$) of plate counts from ready-to-eat foods. When the microbial count is in the range $10^4 - 10^5$ it is tolerable, while $\geq10^8$ is

unacceptable (Roberts, et al., 1996). Therefore, the microbial counts from the watermelons in this study during storage could be regarded as within tolerable limits.

A total of ten microbial isolates were identified during the study. The bacterial isolates included *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Lactobacillus fermenti* and *Staphylococcus aureus*. Fungal isolates included *Aspergillus niger*, *Saccharomyces cerevisiae*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor* sp.

Table 2: Microbial counts across all locations

Parameter	Uselu Market			Oba Market			New Benin Market			
$\times 10^4$ CFU/ml	$\bar{x} \pm$ S.D	Min	Max	$\bar{x} \pm$ S.D	Min	Max	$\bar{x} \pm$ S.D	Min	Max	P-value
Bacterial count at room temperature	4.57±2.45	1.00	6.90	4.67±2.35	1.10	7.10	4.23±2.61	0.19	6.60	P>0.05
Bacterial count at refrigerator temperature	1.63±1.01	0.00	2.70	1.8±1.04	0.00	2.90	1.93±1.14	0.00	3.00	P>0.05
Fungal count at room temperature	2.52±1.25	0.80	4.00	2.88±1.17	1.10	4.30	2.53±1.13	0.60	3.70	P>0.05
Fungal count at refrigerator temperature	1.45±0.92	0.00	2.60	1.75±1.01	0.00	2.80	1.75±1.04	0.00	2.90	P>0.05

Note: P< 0.01 – highly significant; P< 0.05 – significant; P> 0.05 – not significant

Table 2 shows the microbial counts of the samples at the different market locations. Most importantly, the differences in the microbial load on the watermelon samples purchased from the different markets were statistically insignificant (P >0.05). I.e., there was no difference observed in the microbial load of watermelon samples purchased from the different markets.

Table 3: Frequency of occurrence of microbial isolates by storage temperature

Isolate	Frequency at room temperature (%)	Frequency at refrigerator temperature (%)
Staphylococcus aureus	23.8	14.3
Lactobacillus fermenti	14.3	9.5
Bacillus subtilis	23.8	23.8
Enterobacter aerogenes	9.5	0
Pseudomonas aeruginosa	23.8	23.8
Saccharomyces cerevisiae	23.8	19.0
Rhizopus stolonifer	4.8	0
Aspergillus niger	19.0	19.0
Mucor sp.	28.6	4.8
Fusarium oxysporum	23.8	23.8

Table 3 shows the frequency of occurrence of the various microbial isolates at the two storage temperatures. The fungal isolate with the highest occurrence at room temperature was *Mucor* sp, while the bacterial species were *S. aureus*, *B. subtilis* and *P. aeruginosa*. At refrigerator temperature, *F. oxysporum*, *B. subtilis* and *P. aeruginosa* had the highest occurrence. Meanwhile, *R. stolonifer* and *E. aerogenes* were not detected at refrigerator temperature (Table 3). However, in Table 4 some isolates were seen from the first day (day 1) of storage to the last, while others appeared later on during the storage time period. For instance, *P. aeruginosa*, *F. oxysporum* and *B. subtilis* were isolated from day 1 to 5 at both

storage temperatures. *Mucor* sp. was seen at room temperature throughout the storage period, but was only seen at refrigerator temperature on day 1 of storage. *E. aerogenes* was seen at room temperature until day 2, while *L. fermenti* appeared on day 3 at room temperature but was isolated in both storage conditions on days 4 and 5 (Table 4).

Table 4: Incidence analysis of the microbial isolates

Day/Isolate	0	1	2	3	4	5
Staphylococcus aureus	■	■O	■O	■O	■	■
Lactobacillus fermenti	-	-	-	■	■O	■O
Bacillus subtilis	■	■O	■O	■O	■O	■O
Enterobacter aerogenes	■	■	■	-	-	-
Pseudomonas aeruginosa	■	■O	■O	■O	■O	■O
Saccharomyces cerevisiae	-	■	■O	■O	■O	■O
Rhizopus stolonifer	■	-	-	-	-	-
Aspergillus niger	-	-	■O	■O	■O	■O
Mucor sp.	■	■O	■	■	■	■
Fusarium oxysporum	-	■O	■O	■O	■O	■O

■ Present at room temperature; O Present at refrigeration temperature; -Absent in both storage conditions

The microbial isolates represented in this study reflect the sanitary quality during cultivation, harvesting, transportation, storage and processing of the fruit (Allafi and Busamri, 2011). However, they are also indicative of how long the fruit was kept before purchase and sampling (Izah, et al., 2015). *E. aerogenes* is an indicator of fecal contamination whose presence could have been due to contaminated washing water or general unsanitary conditions of the fruit before it was purchased (Izah, et al., 2015). *E. aerogenes* was isolated on day 0 and only persisted at room temperature for 2 days (Table 4). *S. aureus* is part of the normal flora of the skin and its presence could be the result of handling practices (Nwachukwu and Osuocha, 2014). This bacterium was seen at the initial ansalysis (day 0) and persisted till day 3 at both storage temperatures, but was absent from day 4 at refrigerator temperature (Table 4). It is possible these organisms may have been in competition with other microbial species and were thus unable to thrive, or that the refrigeration temperature (4°C) may have inhibited further growth. *P. aeruginosa* and *B. subtilis* are considered part of the normal flora of watermelons (Tambekar and Mundhada, 2006), although *Bacillus* spp. are implicated in food poisoning (Titilayo and Salome, 2014). The presence of *Lactobacillus* signifies the onset of spoilage; hence the isolation of *L. fermenti* from day 3 of storage was not surprising indicating that consumption of the watermelon may be unwholesome because fermentation and deterioration could have commenced, irrespective of the storage temperature. In the study conducted by Nwachukwu and Osuocha, (2014) watermelons were seen to have a higher frequency of microbial isolates than other fruits studied and this could be attributed to the fact that watermelons are grown close to the soil and can easily be contaminated by soil debris. Hence, it is possible that the fungal isolates observed in this study may have originated from soil contamination. Some fungal species such as *Aspergillus, Fusarium* and *Mucor* produce toxins on food products high in carbohydrates. These mycotoxins can have damaging effects when ingested, especially by immunocompromised individuals (Izah, et al., 2015). Generally, *Aspergillus* and *Mucor* species are spore formers

and environmental contaminants, and fruits are suitable habitats for them. Other reports indicate that *Saccharomyces cerevisiae* and molds are contaminants of fruits that have injuries such as slicing (Nwachukwu and Osuocha, 2014). Even though whole watermelon was purchased for this study, there is a possibility that the samples divided at the time of analysis albeit under aseptic conditions may have introduced one or more of the isolated fungal species.

4. CONCLUSION

This study has shown that refrigerator storage (4°C) of watermelon reduces the microbial count compared with room temperature storage (22°C). Indeed, refrigeration can prevent or slow the growth of microorganisms depending on the organisms involved. Essentially, proper storage conditions and effective sanitation are two important factors to consider in preserving the shelf life of fruits. A proactive awareness of and sensitization to the dangers of consumption of contaminated fruit produce subject to either ineffective storage measures or unhygienic practices needs to be available to the public. Also, government agencies should implement policies that check the sale of contaminated fruits and introduce incentives that encourage the application of effective storage measures, which will also preserve the organoleptic quality of fruits for both vendors and consumers.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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