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Original Article

Microbial and biochemical assessment of bacterial and fungal flora of selected fresh fruits sold within Minna

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ABSTRACT

This study focused on identifying and analysing microbial contaminants found on some commonly consumed fruits in Minna, Nigeria. A total of sixty fruit samples were examined, consisting of 20 each of dates, bananas, and watermelons, which were collected from three major markets: Bosso, Kasuwan Gwari, and Central market. Microbial enumeration and isolation were carried out using the pour plate technique on nutrient agar and potato dextrose agar, following standard microbiological protocols. The isolated organisms were identified through cultural observations, Gram staining, and various biochemical tests. Among the fruits, date samples recorded the highest levels of microbial contamination in all three markets, with Bosso market showing the highest bacterial load at 9.2×10^4 CFU/g. Banana samples from Kasuwan Gwari followed closely with a contamination level of 7.2×10^4 CFU/g. Watermelons had the lowest microbial counts, with the highest load being 5.5×10^4 CFU/g, also from Bosso market. Several bacteria were detected in the fruit samples, such as *Bacillus cereus*, other *Bacillus* spp., *Staphylococcus aureus*, *Streptococcus* spp., and *Micrococcus luteus*. The fungi present included *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Penicillium chrysogenum*, and *Mucor* spp. Among the bacterial isolates, *B. cereus* occurred most often (about 45%), followed by *S. aureus* (around 30%), while *Aspergillus* species made up close to two-thirds of the fungal population. These findings point to poor hygiene and handling at various stages—harvest, transport, and sale—as likely sources of contamination. Such conditions raise concerns about the safety of consuming fruits that are

unwashed or poorly handled, emphasizing how urgently sanitary standards in local markets need to be improved.

Keywords: fruits, Bacteria, fungi, Date fruit, Banana, Water melon

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INTRODUCTION

Fruits play an essential role in a balanced diet by supplying vitamins, minerals, dietary fibre, and other nutrients that help maintain good health and lower the risk of disease [1]. Among the most popular fruits worldwide are dates (*Phoenix dactylifera*), bananas (*Musa* spp.), and watermelons (*Citrullus lanatus*), each valued not only for their taste but also for their distinct nutritional profiles and health benefits [2,3,4]. However, despite their dietary importance, fruits are easily exposed to microbial contamination at many points along the production chain—from cultivation and harvesting to processing, transport, and storage [5].

Harmful microorganisms such as bacteria, fungi, and viruses can enter the food supply in numerous ways, and their presence is a major public health concern [6]. Contamination often stems from the use of unsafe irrigation water, inadequate processing facilities, poor storage conditions, unhygienic handling, or cross-contamination during transportation [7]. Because fruits differ in structure and composition, they also differ in how much microbial load they carry. Dates, bananas, and watermelons, for example, show varying levels of spoilage organisms and pathogens. Their naturally high moisture and sugar content can encourage fungal growth and shorten shelf life if not properly managed [8,9].

The health risks from eating contaminated fruit range from mild stomach discomfort to serious systemic infections [5], with vulnerable groups such as children and immunocompromised individuals at greater risk [10]. Some fungi, including species of *Aspergillus*, can produce aflatoxins, dangerous mycotoxins that have been linked to cancer and other severe illnesses [11]. With worldwide fruit intake steadily increasing, it becomes ever more important to keep evaluating the microbial quality of fresh produce, particularly in areas where food safety oversight is limited or inconsistently applied. [12].

Fruits cut by street and market vendors presents an even greater hazard. Removing the peel or rind strips away the fruit's natural barrier, leaving the edible portion exposed to contamination from air, utensils, and human contact [13]. When knives, cutting boards, or the vendor's hands are unclean, harmful bacteria such as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* can easily spread [14]. In addition, cutting increases surface area and releases juice, creating ideal conditions for microbes to multiply rapidly [15]. Numerous studies confirm that sliced fruits carry heavier microbial loads than whole fruits and have been associated with outbreaks of foodborne illness, including gastroenteritis [16].

In many developing countries, including Nigeria, the problem is compounded by unregulated street vending [17]. Addressing these risks will require stricter enforcement of hygiene standards,

targeted training programs for vendors, and broader public education on safe food handling [14]. Conducting studies that identify and characterize the microorganisms on commonly consumed fruits is vital. Such research not only clarifies where contamination originates but also guides policy decisions aimed at keeping fruits safe, nutritious, and fit for human consumption [5].

MATERIALS AND METHODS

Study Area

This research was carried out in Minna metropolis, located within Niger State, which is an area that remains largely rural and less industrialized compared to many other parts of Nigeria. Geographically, the state lies between latitudes 8°20′ N and 11°30′ N and longitudes 3°30′ E and 7°20′ E, placing it in Nigeria's North Central zone. As presented in Figure 1, Niger State is the country's largest by land mass, covering roughly 76,000 km^2 — about 9% of the national total (8.44×10^5 km^2) — partly due to the inclusion of Borgu and Agwara as local government areas. The hottest period typically falls in March and April, just before the first rain, with average daily temperatures often reaching about 32 °C throughout the year.

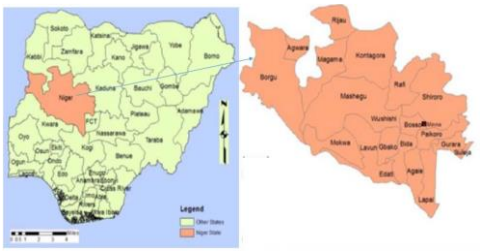

Fig 1: Map of Nigeria, showing Niger State and the local Government areas [19]

Sample collection

Sixty samples of three fruit types (watermelon, banana, and dates) were collected from three markets in Minna, (Bosso, Kasuwan Gwari, and Central market). Each sample was carefully secured in sterile polyethylene bags and promptly transported to the Microbiology Laboratory at the Federal University of Technology, Minna, for analysis.

Isolation of Microorganisms from Fruit Samples

The pour plate method, as described by Cheesbrough (2015), was used to enumerate, isolate, and identify bacterial and fungal species present in fruit samples, including watermelon, date, and banana. For the banana and date fruits, 1 gram of each sample was aseptically weighed and homogenized using a sterile mortar and pestle. In the case of watermelon, 1 millilitre of juice was dispensed into 9 millilitres of sterile distilled water to prepare a 10^{-1} dilution. This was followed by a series of ten-fold serial dilutions up to 10^{-4}.

From the 10^{-3} and 10^{-4} dilutions, 1 millilitre aliquots were transferred aseptically, in duplicates, into sterile Petri dishes. These were mixed thoroughly with approximately 15–20 millilitres of freshly prepared molten nutrient agar (cooled to around 45 °C) to determine the total bacterial count (TBC). For fungal enumeration, 1 millilitre of the 10^{-2} dilution was plated in duplicate and overlaid with molten potato dextrose agar (PDA). All plates were gently swirled to ensure even distribution of the microorganisms throughout the medium.

After the media solidified, incubation of the bacterial plates was carried out at 37 ± 2 °C for 24 hours, while fungal plates were incubated at 28 ± 2 °C, following the method outlined by Douglas [21]. Upon

incubation, colonies enumeration was done using a Coulter counting chamber, and microbial load was calculated using the expression:

Colony Forming Unit (CFU) = Number of Colonies × Volume of Diluent × Reciprocal of Dilution

Purification and Maintenance of Microbial Isolates

Developed bacterial and fungal colonies were randomly selected and purified by sub-culturing onto fresh sterile nutrient agar (NA) and PDA plates. These purified isolates were then transferred to agar slants and stored at 4 °C in a refrigerator as stock cultures for further analysis.

Identification and Characterization of Bacterial Isolates

Bacterial isolates were distinguished through their cultural traits, morphology, and biochemical reactions. The analyses performed included Gram staining, coagulase and catalase assays, carbohydrate fermentation tests using glucose, lactose, and sucrose, as well as citrate utilization, urease activity, methyl red, starch hydrolysis, Voges–Proskauer, and haemolysis tests. Identification relied on comparing these traits with standard reference descriptions provided by Cheesebrough [20].

Gram Staining

Each bacterial sample was smeared onto a clean glass slide, air-dried, and heat-fixed. The slides were stained with crystal violet for about 30 seconds, rinsed under running water, and then treated with Gram's iodine for another 30 seconds. After a second rinse, they were decolorized briefly with alcohol and counterstained with safranin for about one minute. Following a final gentle rinse and blotting with absorbent paper, the slides were examined microscopically. Gram-positive organisms appeared deep purple, whereas Gram-negative organisms showed a red or pink coloration.

Catalase Test

A sterile wire loop was used to transfer a small amount of bacterial colony onto a clean, dry glass slide followed by the addition of a drop of 3% hydrogen peroxide (H_2O_2). The immediate formation of bubbles indicated a positive catalase reaction, while little or no bubbling indicated a negative result [20].

Urease Test

Urea agar slants were inoculated with the bacterial broth cultures, the tubes loosely capped, and then incubated at 37 °C for 48 hours. The appearance of a bright pink to magenta colour signified a positive urease reaction, whereas tubes that showed no colour change were interpreted as negative. [20].

Starch Hydrolysis Test

Nutrient agar supplemented with 0.3% soluble starch was prepared, dispensed into sterile Petri plates, and left to solidify. The bacterial isolates were then streaked across the surface and incubated at 37 °C for 48 hours. Following incubation, each plate was flooded with approximately 5–10 mL of iodine solution. A positive starch hydrolysis reaction was confirmed when a clear zone appeared around the bacterial growth against the blue–black background of the medium. [20].

Citrate Utilization Test

Isolates were inoculated onto Simmon's citrate agar slants and incubated at 37 °C for 24 hours. A positive test was confirmed by the appearance of a deep blue colour on the medium, while no colour change indicated a negative result [20].

Methyl Red Test

Glucose phosphate broth was first prepared and portioned into test tubes, which were then sterilized in an autoclave at 121 °C for 15 minutes. Once cooled, the sterile tubes were inoculated with bacterial cultures and incubated at 37 °C for 48 hours. After incubation, four drops of methyl red indicator were carefully added to each tube and mixed gently. Tubes turning bright red were considered positive for the test, whereas those that remained yellow were interpreted as negative. [20].

Voges–Proskauer Test

To perform the Voges–Proskauer test, 2 millilitres of 40% potassium hydroxide (KOH) and 3 millilitres of 5% alpha-naphthol solution were added to the bacterial cultures grown in peptone water. The mixture was incubated at 37 °C for 48 hours and gently shaken. The appearance of a pink coloration indicated a positive result [20].

Haemolysis Test

Fresh bacterial cultures (24 hours old) were streaked onto blood agar plates and incubated at 37 °C for 24 hours. The plates were then examined for haemolytic activity. Beta-haemolysis was observed as a clear zone of complete red blood cell lysis around colonies. Alpha-haemolysis was characterized by a greenish discoloration surrounding the colonies, indicating partial lysis. Gamma-haemolysis showed minimal to no change in the medium, indicating no haemolytic activity [20].

Identification and Characterization of Fungal Isolates from Fruit Samples

Fungal isolates were identified using both macroscopic and microscopic techniques. Identification was based on cultural features and colony morphology, and findings were compared with known taxonomic references using the classification systems described by Cheesbrough [20].

Cultural Characteristics

Colony features such as pigmentation, texture, and growth patterns were observed during incubation. These characteristics were recorded and used to aid in identification by comparing them to known reference taxa using the methods of Domsch and Gams (as cited in [20]).

Colony Morphology (Microscopic Examination)

A drop of lactophenol cotton blue stain was put on a grease free glass slide, and a small fragment of the fungal mycelium was carefully placed into it. The material was spread out gently to preserve its structure, then covered with a coverslip. The slide was examined microscopically to document and describe the distinctive morphological features of the fungus. [20].

Determination of Frequency of Occurrence of Microbial Isolates

The occurrence rate of both bacterial and fungal isolates was calculated by recording

how frequently each microorganism appeared in the fruit samples. The percentage frequency was calculated using the following expression:

$$\% \text{ frequency of occurrence} = \frac{\text{Number of isolate}}{\text{Total number of islates}} \times 100 \quad [22]$$

RESULTS

Bacterial Load of Fruit Samples

The assessment of bacteria loads across fruits and markets revealed marked differences, as illustrated in Figure 1. Statistical testing confirmed a significant variation among the groups ($P < 0.05$), highlighting distinct patterns of microbial distribution with potential relevance for food safety. At the Central Market, bacterial counts were generally moderate, ranging between 4 and 6 × 10^4 CFU/g across watermelon, banana, and date. Samples from Kasuwan Gwari displayed higher levels, particularly in banana and date, both of which exceeded 7 × 10^4 CFU/g, whereas watermelon remained comparatively lower. The Bosso Market exhibited the greatest contamination, with date recording approximately 9.2 × 10^4 CFU/g and banana also showing elevated values, while watermelon presented intermediate counts.

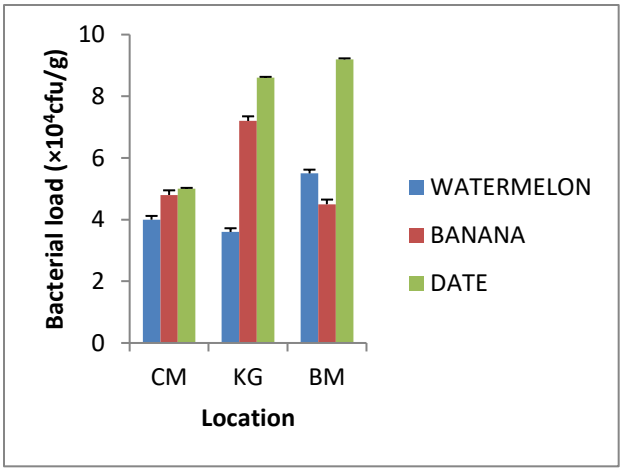

Figure 1 showing Bacterial Load of Fruit samples: CM – Central market, KG- Kasuwan Gwari and BM – Bosso market

Fungal Load of Fruit Samples

The distribution of fungal loads across the sampled fruits also revealed marked variation between markets (Figure 2). In the Central Market, counts were moderate overall, with watermelon presenting higher levels than both banana and date. At Kasuwan Gwari, date carried the highest fungal burden, approaching 9 × 10^2 CFU/g, whereas banana and watermelon showed comparatively lower counts. Bosso Market again recorded elevated levels, with both banana and date exceeding 7 × 10^2 CFU/g, while watermelon remained within the mid-range.

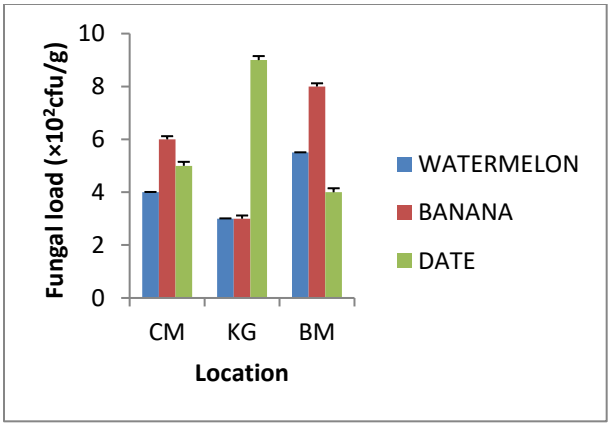

Figure 2 showing Fungal Load of Fruit samples: CM – Central market, KG- Kasuwan Gwari and BM – Bosso market

Frequency of Occurrence of Bacterial Isolates and Fungal Isolates

Among the bacterial isolates identified, *Bacillus cereus* emerged as the dominant species, representing nearly one-third of the total. *Pseudomonas* spp. and *Staphylococcus aureus* were also prominent, each accounting for more than one-fifth of the isolates. In contrast, *Streptococcus* spp. and *Micrococcus luteus* appeared less frequently, contributing only a minor proportion to the bacterial profile as shown in Figure 3.

The fungal isolates displayed a more even distribution across genera as depicted in Figure 4. *Aspergillus flavus* and *Aspergillus niger* were the leading taxa, each recorded in more than a quarter of the isolates. *Aspergillus fumigatus* and *Mucor* spp. also featured strongly, while *Penicillium chrysogenum* was present at a moderate level. The findings reflect a co-occurrence of multiple fungal groups, with species of *Aspergillus* forming the predominant cluster.

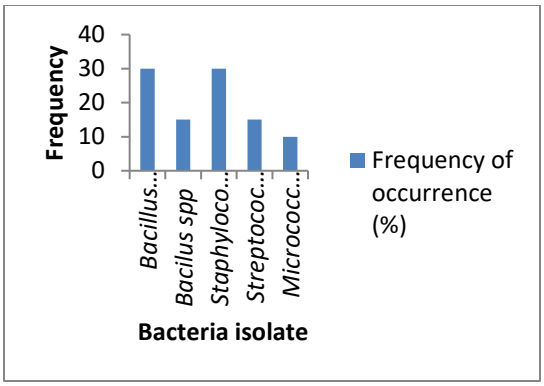

Figure 3 showing Frequency of occurrence of Bacterial Isolate from fruit samples

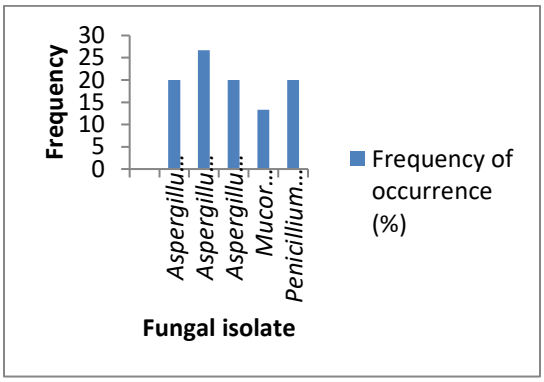

Figure 4 showing Frequency of occurrence of Fungal Isolate from fruit samples

DISCUSSION

Bacterial Load of Fruit Samples

Among all the fruits tested, dates consistently carried the highest bacterial counts. In particular, samples from Bosso market reached 9.2×10^4 CFU/g, with Kasuwan Gwari dates close behind at 8.6×10^4 CFU/g. Similar findings were reported by Raimi [23], who recorded bacterial counts of 8×10^5 to 19×10^5 CFU/mL in date fruits from Owode market in Offa, Kwara State. The tendency for high contamination in dates may be linked to their sticky surface and high sugar levels, which favour microbial growth [24].

Furthermore, traditional harvesting and processing practices often expose dates to open-air conditions for long periods, increasing their microbial load [25].

Bananas displayed a different contamination profile. The highest bacterial load appeared in bananas from Kasuwan Gwari (7.2×10^4 CFU/g), while lower levels were found in those from Central market (4.8×10^4 CFU/g) and Bosso (4.5×10^4 CFU/g). These differences suggest that handling and hygiene practices vary among markets [26], which may have directly affected the microbial quality of the fruit.

Watermelons, by contrast, carried the lowest bacterial counts overall. Bosso market samples recorded 5.5×10^4 CFU/g, whereas Kasuwan Gwari samples showed the lowest level at 3.6×10^4 CFU/g. The relatively better performance of watermelon may stem from its thick rind, which provides natural protection against surface contamination. Because watermelons are typically cut only when purchased, rather than beforehand, there is less time for microbial contamination. Some studies also suggest the rind itself may have mild antimicrobial effects that help limit bacterial growth [27].

Bosso market stood out with higher microbial loads across all the fruits tested. This could be due to several factors, such as poor hygiene among vendors, exposure to dust or flies, improper storage, and the kind of water used for washing fruits. These observations support earlier studies that stress how much vendor behavior and market conditions can affect the safety of fresh produce [28].

It's necessary to note that many of the date and banana samples tested had bacterial loads that went above the recommended safety limit for ready-to-eat fruits ($<10^4$ CFU/g), according to food safety standards [29,30]. This level of contamination could pose a serious risk, especially for vulnerable groups like children, pregnant women, and immunocompromised. Overall, the findings highlight the urgent need for better hygiene practices throughout the fruit supply chain—from harvest to final sale. Solutions could include educating fruit sellers, improve market facilities, and regularly monitoring for microbes [14,31].

Fungal Load of Fruit Samples

Date fruits from Kasuwan Gwari market recorded the highest fungal count at 9.0×10^2 CFU/g, while bananas and watermelons from the same market had the lowest fungal counts, each at 3.6×10^2 CFU/g. These results are in line with findings reported by Michael *et al.* [32], who investigated fungal loads in ready-to-eat fruits sold in Sango Market, Oyo State.

Several factors may explain the high level of fungal contamination observed in some samples. The time of harvest can significantly influence mould presence, as airborne fungal spores are more prevalent during dry and windy seasons. In addition, exposure during post-harvest handling, transport, and open-market display increases the risk of contamination [23]. Fruits are especially vulnerable to fungal colonization throughout the supply chain, including during storage and at the point of sale.

Fungi such as *Aspergillus*, *Alternaria*, and *Penicillium* species thrive in high-moisture environments like fresh dates, particularly during periods of rain or elevated humidity. Even dried dates may

harbour fungal contamination depending on drying techniques, length of exposure, and storage conditions [33]. In tropical and subtropical climates, conditions such as heat and humidity create optimal environments for fungal proliferation, especially by *Aspergillus* and *Penicillium* species [34].

Extensive research supports the persistence and diversity of fungal contamination in dates across multiple regions. In Nigeria, recent work by Adewunmi *et al.* [35] reported high fungal counts in date fruits from Lagos State. Similar findings were recorded in Adamawa by Anjili *et al.* [36], Kwara by Risiquat [37], and Kano by Ibrahim and Rahma [38]. Outside Nigeria, studies such as that by Elsharawy *et al.* [34] in Saudi Arabia also confirmed significant fungal contamination in dates, indicating that this issue is both widespread and persistent across different geographies.

Collectively, the results of this study and others suggest that fungal contamination in date fruits remains a global concern and warrants more stringent control measures throughout production, post-harvest handling, and distribution.

Identity and Frequency of Occurrence of Bacterial and Fungal Isolates from Selected Fruit Samples

The microorganisms found in this study were identified through their cultural appearance, microscopic features, and biochemical behavior. The bacterial isolates included *Staphylococcus aureus*, *Streptococcus* spp., *Bacillus cereus*, *Bacillus* spp., and *Micrococcus luteus*. Fungal isolates consisted of *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Mucor flavus*, and *Penicillium chrysogenum*. These findings are consistent with those reported by Omokanye *et al.* [39], who found similar organisms in pre-cut fruits sold in Ilorin.

Among the bacteria, *Bacillus cereus* and *Staphylococcus aureus* appeared most frequently in all fruit types studied — watermelon, banana, and date. In contrast, *Micrococcus luteus* was found the least often. The breakdown of bacterial frequency across markets is shown in Figure 3.

Interestingly, all bacteria identified in this study were Gram-positive. This contrasts with results from Michael *et al.* [32], who reported a mix of both Gram-positive and Gram-negative organisms. Since species like *Bacillus* are commonly found in the environment — including in soil, on plants, and even on human skin — their presence on fruits might be linked to unclean handling practices, poor hygiene, or the use of contaminated water [7].

Aspergillus species were the most commonly found fungi across the fruit samples. As shown in Figure 4.5, *A. niger* had the highest frequency, accounting for 40% of all fungal isolates, while *Mucor flavus* was the least frequent at 15%. Notably, *A. niger* was present in every fruit sample tested.

The presence of *Aspergillus* and *Penicillium* species on date fruits raises concern, as these fungi are known to produce mycotoxins that can seep into the fruit and may pose health risks when consumed [40]. *Aspergillus fumigatus* — often found in the environment — was also commonly detected. Its presence likely results from post-harvest exposure during handling, processing, or storage [34]. Altogether, these findings suggest

that contaminated fruits could act as carriers of harmful pathogens to consumers

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Authors contribution; F.Sanusi designed research, F.Sanusi and R. Ismaila conducted the research, Y.Idris with A.Usman analyzed the results and wrote the paper. All authors read and approved the final manuscript

Conflict of interest

Authors declare no conflict of interest

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