

Bioburdens of Selected Ready-to-Eat Fruits and Vegetables Consumed in Katsina Metropolis, Katsina State, Nigeria

Mannir Kabir¹, Yahaya Yunusa Riko², Bilkisu Abdullahi³, Kamaluddeen Kabir⁴, Umar Darma Zubairu⁵, Umar Adamu Hamza⁶

¹Department of Microbiology, Umaru Musa Yar'adua University, PMB 2218, Katsina, Nigeria
mannir.kabir[at]gmail.com (Corresponding Author)

^{2, 4, 5}Department of Microbiology, Umaru Musa Yar'adua University, PMB 2218, Katsina, Nigeria

³Department of Biology, Al-Qalam University, PMB 2137, Katsina, Nigeria

⁶Department of Life Sciences, Faculty of Science and Technology, Mewar University, Gangrar, Chittorgarh, Rajasthan, India

Abstract: Ready-to-eat vegetables and fruits have intrinsic properties predisposing them to microbial contamination especially in the absence of quality control. Recently, the increase in consumables' bio-burden, manifested as the accumulation of potential pathogenic microbes therein, trigger occurrence of food related ailments, associated with morbidity and mortality cases. Evaluating the microbial quality of such foods is therefore important. This study was aimed at evaluating the presence of pathogens (bacteria and fungi) associated with some select fruits and vegetables consumed within Katsina metropolis, and comparing their microbial counts against international standards, to ascertain whether they conform to or exceed safety thresholds. Six different fruits and vegetables: cabbage (*Brassica oleracea*), carrot (*Daucus carota*), orange (*Citrus sinensis*), pineapple (*Ananas comosus*), spinach (*Spinacia oleracea*) and watermelon (*Citrullus lanatus*) were sampled from vendors at different selling points. Analyses of the samples were conducted at the Microbiology Laboratory, Umaru Musa Yar'adua University, Katsina, Nigeria. Total Bacteria, Yeast and Mould counts were evaluated according to USDA protocols. The microorganisms were characterized using the colony morphology and biochemical analyses, and comparison with a fungal atlas. The results of the study showed that all the fruits and vegetables invariably contain concentrations of the bacteria exceeding safety thresholds set by the Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, US Academy of Sciences ($p = 0.382$). Nonetheless, from the total yeast and mold counts results, watermelon and carrot have no fungal contaminants, while the rest have concentrations exceeding safety thresholds, with no significant differences ($p = 0.459$). The characterized bacteria include *Enterobacter aerogenes*, *Escherichia coli*, *Proteus* sp., *Shigella* sp., *Staphylococcus aureus* and *Staphylococcus epidermidis*; whereas the characterized fungal isolates were: *Aspergillus flavus*, *Aspergillus niger* and *Saccharomyces cerevisiae*. It is therefore concluded that potentially pathogenic microorganisms exist at high concentrations in the ready-to-eat fruits and vegetables sold in Katsina metropolis which is a reflection of poor hygienic practices. These pathogens may ultimately cause serious illnesses. Hence, we recommend the enshrinement of hygienic processing, educating the vendors and enforcing guidelines by statutory national bodies like Standards Organisation of Nigeria (SON), the National Agency for Food and Drugs Administration and Control (NAFDAC), among others, so as to achieve food safety and maintain the health of the consuming populace.

Keywords: bioburden, microbial pathogens, microbial limits, ready-to-eat foods.

1. Introduction

Vegetables are the edible, leafy parts or seeds of plants used by humans for nutrition purposes like ingredients of soups, parts of a meal, or meals in themselves [1]; however, the matured products of the developed ovaries of flowers which usually bear the seeds are called the fruits [2]. These are regarded as cornerstones of nutrition which provide human body with essential nutrients that facilitate growth and development thereby boosting human immunity [3].

Fruits and vegetables harbor autochthonous microorganisms as part of their epiphytic micro-biota. Similarly, their nature and composition (acid, sugar content and water activity) make fruits and vegetables susceptible to microbial contamination [4, 5]. In the same vein, upon their harvest, poor hygienic and sanitary processing conditions and unhygienic behaviors of the vendors and consumers also

contribute to higher loads of microorganisms before being consumed [6].

Naturally, certain microorganisms on the fruits and vegetables may be pathogenic to the plants themselves and to humans that consume them, thus, they trigger spoilage, manifesting as unwanted changes in the color, odor, and texture, and a times, toxins production [7; 8]. Spoilage of fruits and vegetables is a rampant phenomenon in Nigeria as up to 70% and 20% of the vegetables and fruits produced are spoiled before being consumed [9; 10].

In the United States for example, it has been posited that the consumption of fruit and vegetable products has dramatically increased by more than 30% [6]. However, 20% of all the fruits and vegetables products which are produced are lost each year due to spoilage.

The spectrum of pathogenic microbes associated with fruits include from bacterial species of the genera *Bacillus*, *Clostridium*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Shigella*, while the fungal genera include *Aspergillus*, *Alternaria*, and *Penicillium* [5]. The presence of these microorganisms in food can be debilitating to humans, e.g. through production of toxins like aflatoxins that have carcinogenic potentials [11].

Bacillus sp., *E. coli*, *Klebsiella* sp. and *Staphylococcus* spp. were previously isolated from spoiled banana, papaya and pineapple; while spoilt carrots harbored *S. marcescens*, *E. coli* and *C. bovis*, *P. digitatum*, *R. stolonifer*, *A. niger* and *A. alternata* [1; 12]. Similarly, *Aspergillus* sp. and *Rhizopus* sp. were isolated from pawpaw sold in South-Western Nigeria [13].

In the study area (Katsina metropolis, Katsina State, Nigeria), vegetables and fruits constitute a significant portion of the diet of the people. These diets are continuously consumed mostly without further processing, either singly, or in salads, as such, amplifying the risk of contracting microorganisms from the vegetables and fruits. Moreover, it is a fact that generalized awareness about the prevalence and possible consequences emanating from the presence of pathogens is low. This phenomenon can be attributed to many causes, nonetheless, it makes researches of this kind, seeking to study the prevalence of these pathogenic microorganisms in different localities and establish the prevalence levels, enhance public awareness, and initiate the devising of possible control strategies, imperative. Such a study will also reveal whether or not the ready-to-eat fruits/vegetables are safe for human consumption, upon comparison with internationally set microbiologic standards.

2. Methodology

2.1 Study Area

This study was conducted in Katsina Local Government Area of Katsina State in North-Western Nigeria. Katsina State covers an area 23,938 sq. km. and is located between latitudes 11°08'N and 13°22'N and longitudes 6°52'E and 9°20'E. The state is bounded by Niger Republic to the north, Jigawa and Kano States to the east, Kaduna State to the south and by Zamfara State to the west. It is located at an elevation of 464 meters above sea level, with mean annual maximum and minimum temperatures of 33.2°C and 18.7°C respectively. According to projections by the National Bureau of Statistics, by 2016, Katsina State has an estimated population of 7,831,319; [42] while Katsina metropolis has an estimated population of 429,400 [43]. The climate is said to be Sudan Savannah, and the soil types are predominantly sandy-loam, with clayey soils rarely found [14].

2.2 Study Design and Sample Procurement

Since the study was cross-sectional in design, different areas where fresh fruits and vegetables are sold were used for the study, including: cabbage (*Brassica oleracea*), carrot (*Daucus carota*), orange (*Citrus sinensis*), pineapple (*Ananas comosus*), spinach (*Spinacia oleracea*) and

watermelon (*Citrullus lanatus*). These were purchased from different vendors and packaged aseptically in sterile polythene bags [15]. They were then transported to the Microbiology laboratory, Umaru Musa Yar'adua University, Katsina for further analyses.

2.3 Samples Preparation and Bioburden Evaluation

The protocol of USDA with minor modification was adopted for bioburden evaluation, through the evaluation of Total Aerobic Bacterial Counts and Total Yeast and Mold Count [16; 12].

One gram of the homogenate from each of the ground fruits and vegetable samples were separately diluted with 9mls of the peptone water to obtain the 10^{-1} dilution factor. Further dilutions were made serially up to the 10^{-6} factor [17].

From the 10^{-5} and 10^{-6} dilutions, 100µl aliquots were inoculated separately onto Nutrient Agar and Potato Dextrose Agar Plates for the bacterial and fungal counts using the spread plate technique respectively. The same quantity of the 6th dilution was inoculated onto MacConkey Agar (MA), Eosin Methylene Blue Agar (EMBA), *Salmonella-Shigella* Agar and Mannitol Salt Agar (MSA) for selective isolation of specific potentially pathogenic bacteria. All plates were incubated at appropriate temperatures and times for the bacteria and fungi [17].

Aerobic Mesophilic (Bacterial) Counts of 30-300 were recorded. The results were expressed as average $\pm$ S.D. of the duplicate plates, and were calculated using the formula modified from USDA [15]:

$$\text{CFU/g} = \frac{\text{No of colonies counted}}{\text{Volume of sample plated}} \times \text{dilution factor}$$

Fungal counts were calculated using the same formula, but the colonies were counted with the aid of hand lens, after 5 days incubation. Counts were expressed as average $\pm$ S.D. of duplicate plates, and were reported in CFU/g.

2.4 Characterization of Bacterial and Fungal Isolates

Bacterial isolates were characterized using the three-tiered procedure adopted from Darma *et al.* [18]. First, colonial morphologies of individual colonies on the selective media were recorded. Then, Gram's reaction identifies the cellular morphology, and finally, biochemical characterizations were done. Specie identification was done through comparison with Bergy's Manual of Deterministic Bacteriology and Cowan and Steel's Manual of microbial identification [19; 20]. The biochemical tests conducted on the isolates include the IMViC group of tests, catalase, coagulase, oxidase, urease, sugar fermentation, motility tests, among others [21].

The fungal colonies were identified using colony appearance by observing the texture, pigmentation, color, and size while microscopic observation was carried out using the lactophenol cotton blue staining, for features such as the hyphae type, arrangement, and presence of mycelia/conidia [6]. An atlas was used to aid the identification process [22].

2.5 Data Analysis

Data obtained was analysed using Microsoft Excel Data Analysis ToolPak (2007 version). One-way Analysis of Variance was used to test whether there is a significant difference between the aerobic mesophilic counts/total yeast and mold counts of the various ready-to-eat fruits/vegetables, at 95% confidence interval [23].

3. Results

3.1 Bioburden of the Fruits and Vegetables: Aerobic Mesophilic Counts (AMC) and Total Yeast and Mold Counts (TYMC)

The results of the bio-burden evaluation of the fruits and vegetables showed that the highest counts were obtained in pineapple, carrot, watermelon, orange, cabbage and spinach (Table 1). All the counts exceed international limits sets by the Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, as outlined by National Academy of Sciences (2003), and as such, are designated as unacceptable.

With regards to the results of total Yeast and Moulds Counts, two fruits: watermelon and carrot have acceptable quality, as they have zero colony counts. However, the remaining four fruits/vegetables contained higher counts above the International Microbiologic Standards, as such are classified as unacceptable (Table 2).

Table 1: Aerobic Mesophilic Count of Some Ready-to-Eat Fruits and Vegetables Sold in Katsina Metropolis, and their Safety Status According to International Microbiologic Criteria

S/No	Samples	Average CFU/g ± S.D. ($\times 10^5$)	Status of Food*	P-value
1	Orange	13.9±1.01	Unacceptable	0.382
2	Pineapple	30.5±0.07	Unacceptable	
3	Watermelon	18.9±1.71	Unacceptable	
4	Carrot	21.1±1.83	Unacceptable	
5	Cabbage	8.7±0.33	Unsatisfactory	
6	Spinach	6.1±0.27	Unsatisfactory	

Key: *Status of food material is evaluated by comparison with international microbiologic criteria for safety of fruits and vegetables available at appendix E (National Academy of Science, 2003, p. 354-355, vegetables and vegetable meals. The quality criteria are: borderline safe: 10^4 - 10^5 CFU/g, unsatisfactory = $>10^5$ CFU/g; and ready-to-eat, some components not cooked in manufacturing process, p. 338-339, quality criteria: marginal quality = $\leq 1.0 \times 10^5$, and unacceptable quality = $\geq 5.0 \times 10^5$).

The Single Factor ANOVA calculated P-value was 0.382, where $p \leq 0.05$ is considered significant. This shows that there is no significant difference between the colony counts observed from the different fruits and vegetables tested. This is reflected in the fact that all the samples had unacceptable/unsatisfactory quality, exemplified by their high colony counts.

3.2 Bacteria and Fungi Identified from the Fruits and Vegetables

The overall percentage prevalence of the bacteria and fungi isolated from the fruits and vegetables was presented in Figure 1. The identified bacteria were: *Shigella* sp. with 5% prevalence, *E. coli*, *Proteus* sp. and *S. epidemidis*, with 15% each, *E. aerogenes* (20%) and *S. aureus* (30%). Three fungal species were characterized: *S. cerevisiae*, with 50% prevalence, followed by *A. flavus* with 37.5%, and *A. niger*, which had 25% prevalence.

Table 2: Total Yeast and Mold Counts of Some Ready-to-Eat Fruits and Vegetables Sold in Katsina Metropolis, and their Safety Status According to International Microbiologic Criteria.

S/No	Samples	Average CFU/g ± S.D. ($\times 10^5$)	Status of Food*	P-value
1	Orange	0.9±0.21	Unacceptable	0.459
2	Pineapple	13.6±13.36	Unacceptable	
3	Watermelon	0.0±0.00	Acceptable/Excellent	
4	Carrot	0.0±0.00	Acceptable/Excellent	
5	Cabbage	6.1±4.10	Unsatisfactory	
6	Spinach	15.4±12.16	Unsatisfactory	

Key: *Status of food material is evaluated by comparison with international microbiologic criteria for safety of fruits and vegetables available at (National Academy of Sciences, 2003, p. 332-333, frozen fruits, where the quality criteria are: ≤ 100 is acceptable, and ≥ 100 is unacceptable; and salads and similar, p. 352-353, with quality criteria of: $<10^4$ = satisfactory, $>10^4$ = unsatisfactory).

The Single Factor ANOVA calculated P-value was 0.459, where $p \leq 0.05$ is considered significant. This shows that when the TYMCs of the watermelon and carrot samples are excluded, there is no significant difference between the colony counts of the remaining fruits and vegetables. This may be due to the fact that all the remaining samples had unacceptable/unsatisfactory quality and high colony counts.

3.3 Bacteria and Fungi Identified from the Fruits and Vegetables

The overall percentage prevalence of the bacteria and fungi isolated from the fruits/vegetables was presented in figure 1 below. In increasing order of prevalence, the identified bacteria were: *Shigella* sp. (5%), *Escherichia coli*, *Proteus* sp. and *Staphylococcus epidemidis*, (15% each), *Enterobacter aerogenes*, (20%) and *S. aureus* (30%). For the fungi, three were identified: *Saccharomyces cerevisiae*, which was the most prevalent (50%) followed by *Aspergillus flavus* (37.5%), with *A. niger* (25%) having the lowest prevalence.

The occurrence of the bacteria and fungi in the respective fruits and vegetables was presented in table 3 below. The table showed the most contaminated sample was spinach, wherein 7/9 (77.78%) of the isolates occurred, (five out of six bacteria and two out of three fungi), followed by orange and cabbage (6/9 or 66.67%, each), pineapple and carrot (4/9, or 44.44%, each). The least contaminated was

watermelon, where only *S. aureus* occurred, which also occurred in all the samples. Amongst the fungi, *Saccharomyces cerevisiae* occurred in all samples except carrot and watermelon.

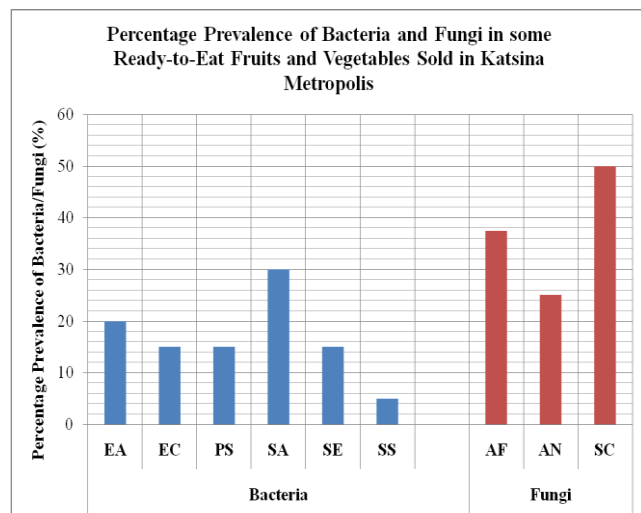


Figure 1: Percentage prevalence of identified bacteria and fungi from some ready-to-eat fruit and vegetables sold in Katsina metropolis

Key: Bacteria: EA = *Enterobacter aerogenes*, EC = *Escherichia coli*, PS = *Proteus sp.*, SS = *Shigella sp.*, SA = *Staphylococcus aureus* and SE = *Staphylococcus epidermidis*. Fungi: AF = *Aspergillus flavus*, AN = *Aspergillus niger*, and SC = *Saccharomyces cerevisiae*.

Table 3: Occurrence of Bacteria and Fungi in some Ready-to-Eat Fruits and Vegetables Sold in Katsina Metropolis

S/No	MO	O	CA	P	CB	W	S
1	Bacteria	EA	●	●	●	●	●
2		EC	--	--	●	●	●
3		PS	●	●	--	--	●
4		SS	--	--	--	--	●
5		SA	●	●	●	●	●
6		SE	●	●	--	--	●
7	Fungi	AN	--	--	●	●	--
8		AF	●	--	●	●	●
9		SC	●	--	●	●	●

Key: MO = Microorganisms (Present and Identified). Fruits and Vegetables: O = Orange, CA = Carrot, P = Pineapple, CB = Cabbage, W = Watermelon, S = Spinach. Bacteria: EA = *Enterobacter aerogenes*, EC = *Escherichia coli*, PS = *Proteus sp.*, SS = *Shigella sp.*, SA = *Staphylococcus aureus* and SE = *Staphylococcus epidermidis*. Fungi: AF = *Aspergillus flavus*, AN = *Aspergillus niger*, and SC = *Saccharomyces cerevisiae*.

● = Present, -- = Absent

4. Discussion

The study evaluated the bioburden of ready-to-eat fruit and vegetables sold in Katsina Metropolis, and isolated and identified the potentially pathogenic bacteria and fungi therein. It is a well-established fact that bacteria and fungi are the commonest contaminants of our fruit and vegetables that are easily transferrable from the vendors to the

processed fruits through mishandling; and the consumption of contaminated, ready-to-eat fruits directly from street vendors or hawkers potentially increases the risk of food-borne diseases caused by a wide variety of pathogens [24]. Ascertaining the hygiene of vendors of those foods, or the microbiologic suitability or lack thereof of the sanitary conditions at points of processing of the fruit and vegetables, as well as the microbial quality of the packaging materials, where they are used, is a burdensome task that is not commonly undertaken, but this could pose a threat to human health (Hasan and Zulhikar, 2018).

The results of the bioburden evaluation from this study are higher than those reported from Maiduguri, which ranged from 1.5×10^5 to 6.0×10^5 CFU/g, whereas in this study, the bacterial bioburden ranged from 6.1×10^5 to 30.5×10^5 CFU/g [25]. The authors described their bioburden as 'high', and attributed it to the [poor] sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce.

From the six (6) different fruit and vegetables microbiologically examined in this study, a total of nine (9) microorganisms were isolated, six (6) bacteria (*Enterobacter aerogenes*, *Escherichia coli*, *Proteus sp.*, *Shigella sp.*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and three (3) fungi (*Aspergillus flavus*, *Aspergillus niger* and *Saccharomyces cerevisiae*). The identity of the microbes isolated and identified from this study mimics those of [26], as reported in their study on the microbial quality of pre-cut fruits sold in Ilorin, Kwara state with the exception of *Shigella sp.* and *A. flavus*, whom they did not isolate/identify. Similarly, the results of our study are in line with the report of [27], who isolated microorganisms from air-contaminated vended foods sold in Lokoja, Kogi state. Contrastively, other studies reported the isolation of *Salmonella sp.* and *Pseudomonas sp.*, which weren't identified in this research. Such studies include the work of [28], which reported the isolation of *Staphylococcus aureus*, *Pseudomonas sp.*, *Salmonella sp.* and *Escherichia coli* from ready-to-eat fruit sold in Otta, Ogun state; and [29], who also isolated *Staphylococcus aureus*, *Pseudomonas sp.*, *Salmonella sp.* and *Escherichia coli* from street vended fruits juices in Amravati, India. These similarities may indicate that a virtually known, common syndicate of microorganisms is responsible for the spoilage of fruit and vegetables.

Most of the isolates in this study, especially the bacterial isolates, most of which belong to the Enteric Gram Negative Rods (*Enterobacteriaceae*) may have been introduced into these fruits through faecally polluted water used in washing utensils like knives, trays and polythene bags used for the packaging of the fruit and vegetables after slicing or cutting and also exposure of these fruit and vegetables to low temperatures which encourage the microbial growth of these pathogens [30]. These isolates, and additionally, the *Staphylococcus aureus* and *S. epidermidis* and all the fungal isolates, are environmental isolates that have repeatedly been isolated from plants, human skin, animal and dairy products. Their presence in these ready-to-eat fruit and vegetables can thus be hypothesized to be from the unclean hands of the vendors, contact with sewage and contaminated water [31].

This implies that the fruit samples could serve as a vehicle in the transmission of these pathogens to the consumers of these contaminated fruits.

The findings of this research are also in line with the findings of [30], who researched on the microbial quality of sliced fresh fruits sold in Minna metropolis. *Escherichia coli* is regarded as primary indicator for microbiological quality of food and water, and its relatively high prevalence shows that these fruits are not safe for human consumption. *Escherichia coli* is mainly thought to be transmitted through faecally contaminated food or water [32]. The high occurrence may have occurred in the contact of contaminated water with the fruits during washing of the fruits and also the inadequate washing of hands by the fruit vendors [29]. Some of these vendors get their water from impure sources; likewise, they sometimes use very little quantity of water to wash or rinse all the fruit and vegetables, which may facilitate cross-contamination. The low occurrence of *Shigella* sp. in this study was also reported by [27] in the assessment of air-contaminated, vended foods sold in Lokoja, Kogi state.

The presence of *Staphylococcus aureus* may have been introduced into the ready-to-eat fruits through body contact of vendors with the fruits because the organism is a normal flora of the nasal passage, hands and skins of healthy individuals [33]. The fact that *S. aureus* was the most prevalent bacteria identified from the samples in this research coincides with the findings of [26], who also reported *S. aureus* as the bacterium with the highest occurrence in fruit. These findings can be attributed to environmental and human contamination, e.g. the use of polyethene bags for the packaging of these fruit and vegetables after being sliced or cut [34]. The significance of the isolation of *S. aureus* in fruits was described by [35], who stated that *Staphylococcus aureus* isolated from fruits caused poisoning and that proliferation of the organisms could lead to food intoxication.

Regarding the fungi identified from the current study. Likewise, [36], while researching the fungal pathogens associated with the spoilage of orange sold in Benin, Edo State, identified *A. niger* as one of the spoilage agents. However, the findings are different from those of [2], who identified *Aspergillus niger* complex, *Aspergillus flavus*, *Fusarium* sp., *Mucor racemosus*, *Mucor* sp., *Rhizopus stolonifer* and *Penicillium chrysogenum* in spoilt fruit and vegetable samples from Wuse, Abuja, Nigeria.

The occurrence of the fungi may be because of the ubiquity of the fungus, and its tendency to promote contamination of fresh vegetables, fruits, etc. Fungi are also able to withstand high concentration of sugar and they can survive in the absence of water or moisture. Moreover, the occurrence of the fungi may be as a result of the exposure of these ready-to-eat fruit and vegetables to dusty or muddy areas, where fungal spores readily abound. Furthermore, the microbial load on leafy vegetables and fruit increase with time during storage, and hence, when they are stored at inappropriate temperatures, they tend to attain temperatures that are suitable for the microbial growth of these pathogens to cause diseases when ingested [37, 38].

The presence of those fungi in the ready-to-eat fruit and vegetables may also be from knives used for cutting or slicing, improper human handling and processing, tables or trays used during peeling and cutting, rinsed water, washing buckets and packaging materials as these fruits are cut, washed, wrapped with transparent polyethene bags and sold to the consumers.

The potential health implications of presence of *Aspergillus* in foods include Aspergilloses of the lung and hypersensitivity reactions, e.g. Otomycosis of the ear [2]. *A. flavus* is known to elaborate Aflatoxin, a mycotoxin with potent carcinogenic potentials, which has been directly correlated with adverse health effects, such as liver cancer, in many animal models [39]. The fungus is also associated with wound infections, keratitis, chronic granulomatous sinusitis, cutaneous aspergillosis, and osteomyelitis upon trauma and inoculation [4]. *A. niger* is also known to elaborate a nephrotoxin called Ochratoxin A [40]. Moreover, *Aspergillus* spp. are known to produce several toxic metabolites such as malformins and nathopyrones [41]. The isolation of *Saccharomyces cerevisiae* from the samples in this study is in congruity with the findings of [24], who also isolated *Saccharomyces* sp. from spoiled fruits sourced at Gwagwalada, Abuja, Nigeria.

The presence of these potential pathogens in the analyzed fruit samples should be of great importance to the vendors and consumers themselves, and also the concerned governmental agencies.

5. Conclusion and Recommendations

The bacteriologic bioburden of the ready-to-eat fruit and vegetables sold in Katsina metropolis ranged from 6.1×10^5 to 30.5×10^5 CFU/g, and all the samples contain AMCs exceeding safety thresholds set by the Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food of the US National Academy of Sciences. Four samples: spinach, cabbage, orange and pineapple also contain TYMCs exceeding safety limits set by the Committee, with their bioburden ranging from 0.9×10^5 to 15.4×10^5 CFU/g. Only watermelon and carrot samples contained no fungal contaminants whatsoever. Although the microorganisms isolated and identified from this study (*Enterobacter aerogenes*, *Escherichia coli*, *Proteus* sp., *Shigella* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Aspergillus flavus*, *Aspergillus niger* and *Saccharomyces cerevisiae*) are commonplace in the environment, water, soil and on humans, their persistence and proliferation at such high concentrations reported by this study is a reflection of poor hygienic practices by the sellers of the raw fruit and vegetables. We hereby submit that potentially pathogenic microorganisms exist at concentrations exceeding safety thresholds in ready-to-eat fruit and vegetables sold in Katsina metropolis, Katsina state, Northwestern Nigeria, which have the prospect of triggering outbreaks of food-borne diseases. We therefore recommend that hygienic techniques shall be employed for the preparation and processing of the foods to reduce the

bioburden to acceptable limits, and to possibly eliminate the microbial contamination of the final product. Such hygienic techniques shall encompass personal hygiene on the part of the vendors of the fruit and vegetables themselves, use of clean water for washing and cleaning the fruit and vegetables, adequate disinfection of the utensils used for preparing the fruit and vegetables, among others. Finally, government agencies, such as NAFDAC and SON must adopt measures to educate the vendors about food safety and hygienic practices and enforce adequate guidelines for ready-to-eat fruit and vegetables in Nigeria, towards safer ready-to-eat fruits/vegetables, and enhanced health of the consuming populace.

References

- [1] O. Samuel, N. Ogonna, O. Ifeanyi, O, "Isolation, Characterization and Identification of Microorganisms from Spoilt Carrots Obtained from Ose Market Onitsha, Nigeria", Universal Journal of Biomedical Engineering, 4 (1), pp 6-9, 2016.
- [2] E.K.. Onyemata, R.O. Ibrahim, "Isolation and Identification of Fungi and Pathogenicity Assessment of some Spoilt Fruits Sold in Wuse Market, Abuja, Nigeria", International Journal of Current Research, 10 (12), pp 76256-76259, 2018.
- [3] P.U. Iniekong, I.E. Clara, O.O. Bryan, E.O. Martin, (2015). "Fungi Responsible for the Spoilage/Deterioration of Some Edible Fruits and Vegetables", Advances in Microbiology, 5 (4), pp 285-290.
- [4] M.T. Hedayati, A.C. Pasqualotto, P.A. Warn, P. Bowyer, D.W. Denning, D.W., "Aspergillus flavus: human pathogen, allergen and mycotoxin producer", Pulmonary Medicine, 153 (Pt6), pp 1677-1692, 2007.
- [5] J. Wiley, L.M. Sherwood, C. Woolverton, Prescott's Microbiology, McGraw-Hill Professional Publishers, New York, 2013.
- [6] M. Barth, T.R. Hankinson, H. Zhuang, H. Breidt, "Microbiological spoilage of fruits and vegetables," in Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety, W. H. Sperber and M. P. Doyle, (eds.), Springer Science and Business Media, Berlin, 2009.
- [7] S. Mailafia, G.R. Okoh, H.O.K. Olabode, R. Osanupin, "Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria", Veterinary World, 10 (4), pp. 393-397, 2017.
- [8] J.L. Richard, "Some major mycotoxins and their mycotoxins: an overview", International Journal of Food Microbiology, 119 (1-2), pp. 3-10, 2007.
- [9] B. Thiyam, G.D.Sharma, "Isolation and identification of fungi associated with local fruits of Barak Valley, Assam", Current World Environment, 8 (2), pp. 319-322, 2013.
- [10] D. Singh, R.R. Sharma, "Postharvest diseases of fruit and vegetables and their management", in Sustainable Pest Management, D. Prasad, (ed.), Daya Publishing House, New Delhi, 2009.
- [11] International Agency for Research on Cancer – IARC, "Monograph on the evaluation of carcinogenic risks to humans, some naturally occurring substances: some traditional herbal medicines, some mycotoxins, naphthalene and styrene", IARC Monographs, 82, pp 171-300, Lyon: IARC, 2002.
- [12] N.A. Hasan, I.M. Zulkahar, "Isolation and identification of bacteria from spoiled fruits", In Proceedings of the AIP Conference 2020, Advances in Civil Engineering and Science Technology: AIP Conference Proceedings 2020, pp. 020073-1-020073-5, 2018.
- [13] R.A. Baiyewu, N.A. Amusa, O.A. Ayola, O.O. Babalola, O.O. "Survey of the postharvest diseases and aflatoxin contamination of marketed pawpaw fruits (*Carica papaya*) in South-Western, Nigeria", African Journal of Agricultural Research, 2 (4), pp. 178-181, 2007.
- [14] Y. Abdulhamid, M.S. Abdulsalami, A.B. Sulaiman, I.K. Matazu, "Determination of Physicochemical and Some Heavy Metal of Soil Around DANA Steel Industry Limited, Katsina, Katsina State, Nigeria", UMYU Journal of Microbiology Research, 2, pp. 15-20, 2017.
- [15] Z. Yusuf, O.M. Oyaweye, K.A. Yongbi, A.T. Pemu, "Bacteriological Quality Assessment of Salad Vegetable Sold in Bauchi Metropolis", Nigerian Journal of Microbiology, 18, pp. 316-320, 2010.
- [16] United States Department of Agriculture, USDA, "Standard Operating Policy/Procedure: Standard Bacterial Plate Count", Ames, Iowa: Animal and Plant Health Inspection Section, USDA, 2015.
- [17] M. Cheesbrough, District Laboratory Practice for Tropical Countries. Part II, Cambridge University Press, Cambridge, 2006.
- [18] U.Z. Darma, A.Z. Mansir, Y.Y. Riko, "Compatibility and Formulation of Diesel Degrading Consortia Using Bacteria Isolated from Contaminated Soil", Bayero Journal of Pure and Applied Sciences, 12 (1), pp. 199-208, 2019.
- [19] P. de Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.H. Schleifer, W.B. Whitman, (eds.), Bergy's Manual of Systematic Bacteriology, Volume III, The Firmicutes, Springer, Georgia, 2003.
- [20] G.I. Barrow, R.K.A. Feltham, Cowan and Steel's Manual for the Identification of Medical Bacteria, Cambridge University Press, Cambridge, 2004.
- [21] V. Hemraj, S. Diksha, G. Avneet, "A Review on Commonly Used Biochemical Tests for Bacteria", Innovare Journal of Life Sciences, 1, PP. 1-7, 2013.
- [22] L.A. Omar-Zahid. Atlas of Food Microbiology Lab: Microorganisms Including Bacteria, Molds and Yeasts. [E-Book], Department of Biology, University of Baghdad, Iraq, 2013.
- [23] K.N. Berk, P. Carey, Data Analysis with Microsoft Excel Updated for Excel 2007. Brooks/Cole, Cengage Learning, Massachusetts, 2010.
- [24] S. Mailafia, G.R. Okoh, H.O.K. Olabode, R. Osanupin, "Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria," Veterinary World, 10 (4), pp. 393-397, 2017.
- [25] I.A. Allamin, M.B. Borkoma, M. Ibn Abbas, M.A. Waziri, "Bacteriological Quality of Sliced Fruits Sold at Road Site in Maiduguri, Borno State", International Journal Of Environment, 4 (2), pp. 236-242, 2015.

- [26] M.B. Odebisi-Omokanye, M.A. Oke, A.A.M El-Imam, A.K. Ajijolakewu, B.I. Salaudeen, "Microbiological Quality and Safety of pre-cut fruit retailed in Ilorin, Kwara State, Nigeria", Fountain Journal of Natural and Applied Sciences, 4 (1), pp. 19 – 26, 2015.
- [27] P.T. Fowoyo, "Microbiological assessment of air contamination of vended foods sold in the main market in Lokoja, Kogi state, Nigeria", Research Journal of Biological Sciences, 7 (9-12), pp. 355-360, 2012.
- [28] S. Oranusi, O.J. Olorunfemi, "Microbiological safety evaluation of street vended ready-to-eat fruits sold in Ota, Ogun state, Nigeria", International Journal of Biological Science, 1, pp. 27-32, 2011.
- [29] D.H. Tambekar, V.J. Jaiswal, D.V. Dhanorkar, P.B. Gulhane, K. Dudhane, "Microbial quality and safety of street vended fruit juices: A case study of Amravati city", International Journal of Food Safety, 10, 72–76, 2009.
- [30] S.Y. Daniyan, C.Q. Ajibo, "Microbiological examination of sliced fruits sold in Minna Metropolis", International Research Journal of Pharmacy, 2 (7), pp. 124-129, 2011.
- [31] C. de Rover, "Microbial safety evaluations and recommendation on fresh produce", Food Control, 9 (6), pp. 321-347, 1998.
- [32] G.F. Brooks, K.C. Carroll, J.S. Butel, S.A. Morse, T.A. Mietzner, Jawetz, Melnick and Adelberg's: Medical Microbiology, McGraw-Hill/Lange Medical Publishers, New York, 2013.
- [33] E.W. Nester, D.G. Anderson, C.E. Roberts, N.W. Pearsall, M.T. Nester, Microbiology: A Human Perspective, McGraw Hill Plc, New York, p. 590, 2010.
- [34] C.L. Little, R.T. Mitchell, "Microbiological quality of pre-cut fruits, sprouted seeds and unpasteurized fruit from retail and production premises in UK," Public Health, 7 (3), pp. 567-570, 2004.
- [35] A. Bukar, A. Uba, T.I. Oyeyi, "Occurrence of some enteropathogenic bacteria in some minimally and fully processed ready-to-eat foods in Kano metropolis, Nigeria," African Journal of Food Science, 4 (2), pp. 032-036, 2010.
- [36] F.E. Oviasogie, A.G. Ogofure, A. Beshiru, J.N. Ode, F.I. Omeje. Assessment of fungal pathogens associated with orange fruit spoilage sold in Benin metropolis, African Journal of Microbiology Research, 9 (29), pp. 1758-1763, 2015.
- [37] F.L. Bryan, M. Jermini, R. Schmitt, E.N. Chilufya, M. Mwanza, A. Matoba, E. Mfume, H. Chibiya, "Hazards associated with holding and reheating foods at vending sites in a small town in Zambia", Journal of Food Protection, 60, pp. 391-398, 1997.
- [38] O.K. Muinde, E. Kuria, "Hygienic and safety practices of vendors of street foods in Nairobi, Kenya", African Journal of Food, Agriculture and Nutritional Development, 5, pp. 1-18, 2005.
- [39] M.L. Martins, H.M. Martins, F. Bernardo, "Aflatoxins in spices marketed in Portugal", Food Additives and Contaminants, 18 (4), pp. 315–319, 2001.
- [40] M.L. Abarca, M.R. Bragulat, G. Castellá, F.G. Cabañes, "Ochratoxin A production by strains of *Aspergillus niger* var. *niger*", Applied and Environmental Microbiology, 60 (7), 2650–2652, 1994.
- [41] J.C. Frisvad, R.A. Samson, "Mycotoxin produced by species of *Penicillium* and *Aspergillus* occurring in cereals", in Cereal Grain. Mycotoxin, Fungi and Quality in Drying and Storage, J. Chelkowsk (ed.), Amsterdam, Netherlands, 1991.
- [42] National Bureau of Statistics, NBS. "2017 Demographic Statistics Bulletin", Abuja: FCT, NBS, 2018, <https://nigerianstat.gov.ng/download/775>
- [43] City Population, "Katsina (State, Nigeria) - Population Statistics, Charts, Map and Location, on March 21, 2016". Oldenburg, Germany, [citypopulation.de, https://www.citypopulation.de/php/nigeria-admin.php?adm1id=NGA021](https://www.citypopulation.de/php/nigeria-admin.php?adm1id=NGA021)

Author Profile



Mannir Kabir has a diploma in Science Laboratory Technology from the National Institute of Science Laboratory Technology, in 2011, a Bsc. (Hons) in Microbiology from Umaru Musa Yar'adua University, Katsina, in 2018, and is currently pursuing a Masters Degree in Food and Industrial Microbiology, from the same institution. For the past 11 years, Mr. Kabir had been a Technologist at the Department of Microbiology Laboratory, Umaru Musa Yar'adua University, Katsina, conducting practicals for undergraduate students and assisting in various researches on food/ medical/ pharmaceutical microbiology, biofuels and renewable energy.