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Microbiological Quality Assessment of Ready to Eat Vegetables Sold in Yankaba Market Kano, Kano State Nigeria

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Abstract
Safety of food is still a public health challenge globally. Fresh vegetables can be contaminated with pathogenic microorganisms from farm to table. This study evaluates the microbiological quality of ready to eat vegetables sold in Yankaba market, Kano. A total of forty (40) vegetables (cabbage, carrot, cucumber and lettuce) were collected from Yankaba Kasua and Kasua Turawa, and transported to the laboratory under aseptic condition to determine the bacterial load and fungal loads, identification of bacteria and fungi found in ready to eat vegetables using standard protocols. All samples collected had varying level of bacterial contamination ranging from 1.32x10^{5}CFU/ml to 1.45x10^{6}CFU/ml. The highest mean fungal count was observed in cabbage samples obtained from Yankaba Kasua (8.8x10^{3}MC/ml) and carrot from Kasua Turawa (8.0x10^{3}MC/ml) whereas the least fungal count was observed in lettuce sample obtained from Kasua Turawa (3.2x10^{3}MC/ml). Escherichia coli and Staphylococcus aureus were the predominant bacterial isolates accounting for 28.75% and 23.75% respectively whereas Shigella species and Enterobacter species were the least bacterial specie identified from this study, no Salmonella was isolated from this study. Aspergillus genera was found to be the most common fungi specie identified in all the samples accounting for 58.46% of the fungi identified and Geotrichum species was the least fungi identified. This study clearly shows ready to eat vegetables harboured high microbial load majorly E. coli, S. aureus and Aspergillus species which could pose a potential hazard to consumers. There is an urgent requirement for good microbiological practice in handling of ready to eat vegetables, hence the need for application of critical control point of washing in brine and vinegar during preparation.

Keywords: Vegetables, Contamination, *E. coli*, *Aspergillus* species, Yankaba Market,

INTRODUCTION

Fruits and vegetables are an important part of the human diet and are good sources of vitamins, minerals, phytochemicals, dietary fibre and antioxidants which are beneficial to human health by reducing the risk of diseases (Jhee *et al*., 2019; Balali *et al*., 2020; Alegbeleye *et al*., 2022). The awareness of fruits and vegetable consumption globally is on increase exponentially. FAO recommends consuming at least 400 grams of fruits and vegetables per day (FAO, 2015). Two servings of fruits and three servings of vegetables per day is recommended for adults (Wallace *et al*., 2019). They are an important portion of a balanced diet (Yafetto *et al*., 2019; Feroz *et al*., 2020) with vital role in reducing the risk of some illnesses such as diabetes, obesity, heart diseases and cancer such as colorectal cancer (Weldezgina and

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Muleta 2019; Balali *et al*., 2020). Human infections associated with consumption of raw vegetables is on increase during the past decades due to increase in consumption generally as a result of awareness of the health benefits of consuming vegetables as a part of balanced diet (Faour-Klingbeil *et al*., 2016; Mritunjay and Kumar, 2017).

Microorganisms can contaminate vegetables at any point during harvest and post-harvest which often causes spoilage of food and food poisoning for human when consumed (Harding *et al*., 2016). Raw vegetables are usually consumed as staple food in many parts of Nigeria wherein they are increasingly recognised as an important vehicle for transmission of pathogenic microorganisms that cause food borne diseases (Balali *et al*., 2020; Arienzo *et al*., 2020).

Ready to eat vegetables could be contaminated with various groups of microorganism such as bacteria and fungi which may pose health risk (Łepecka *et al*., 2022). A wide range of vegetables had been implicated in human illness globally, certain ready to eat vegetables are more frequently linked to contamination (Denis *et al*., 2016). Based on the background information, this study was conducted with a view to generating data on the microbiological quality of ready to eat vegetables in the study area so to understand its current burden coupled with implementing policy to reduce contamination of ready to eat vegetables to ensure safety of vegetables consumed so as to prevent associated health risks.

MATERIALS AND METHODS

Study Area

This study was conducted in Yankaba market Nassarawa local government area of Kano state. It is the major vegetable market in Kano. The vegetable market is segmented into Yankaba Kasua and Kasua Turawa. Yankaba Kasua provides services to people of all classes whereas Kasua Turawa provides services to middle class, upper class and foreigners in need of fresh vegetables.

Sample Collection and Transportation

A total of forty (40) vegetables (n=10 each of cabbage, carrot, cucumber and lettuce) were collected from Yankaba Kasua and Kasua Turawa in Yankaba market, Nassarawa local government area of Kano. The samples were collected in sterile zip lock polyethylene bags and transported in a cool box to the laboratory of Nigerian Stored Products Research Institute (NSPRI), Kano for further processing. However, samples were processed within one hour of sample collection.

Sample Preparation

The samples were prepared according to the method described by Arienzo *et al*. (2020). Briefly, ten (10) g of each vegetable sample were weighed, washed under running tap water and then blended in 100 ml of sterile distilled water under sterile conditions. The blender was carefully disinfected using vinegar to prevent any cross contamination. The homogenates were collected in sterile bottles. Aliquot of 1ml from the homogenate were dispensed into 9 ml of sterile distilled water to make the stock solution.

Estimation of Bacterial and Fungal Load

Aliquot of 1 ml from the stock solution was serially diluted in 9ml of sterile distilled water up to 10^{-6}. The bottle containing dilution factor of 10^{-4} were plated onto sterile Nutrient agar (Hi-Media, India), incubated aerobically at 37 °C for 18-24 hours to determine the total aerobic bacterial count. Enumeration and identification of bacterial species was carried out by plating aliquot of 1ml from 10^{-4} tube onto plate of sterile MacConckey agar (Hi-Media, India), *Salmonella Shigella* agar (Hi-Media, India) and were incubated aerobically at 37 ^{o}C for 18-24hours. Distinct colony were sub-cultured onto a new plate of sterile MacConkey agar and incubated at 37 ^{o}C for 18-24 hours for biochemical identification and microscopy. The bacterial growths were subjected to Gram staining, catalase test, coagulase test, urease test, oxidase test, Indole production, Citrate utilization tests, triple sugar Iron Agar (TSI), Oxidase and MRVP to determine the identity of the bacteria. The bacteria were identified using Koneman Chart (Procop *et al*., 2017).

Fungi load determination was carried out according to the method described by Feroz *et al*. (2016). Briefly, 1ml aliquot from 10^{-3} tube was plated onto potato dextrose agar (Hi-Media, India) and subsequently incubated at room temperature. The growth on PDA was counted after 2 days of incubation and expressed as mould count per millilitre (MC/ml). The fungi species were identified after seven (7) days of incubation using physical observation and Lactophenol cotton blue staining technique and interpreted with the aid of Atlas of Mycology.

Statistical Analysis

Data obtained were analysed using Excel 2013. Descriptive statistics was used to calculate the mean microbial load and student T-test to compare the difference between mean bacterial and fungal counts of samples collected from Yakaba Kasua and Kasua Turawa.

RESULTS AND DISCUSSION

Fresh vegetable are inherently contaminated by microorganisms majorly bacteria and fungi (Qadri *et al*., 2015). However, the presence of this bacteria and fungi may be due to the cultivation practices, handling practices at harvest, transportation practices and handling in the market space. Bacteria and fungi are ubiquitous in nature, abound in soil, air, water, surfaces and on the hand of handlers. Cultivation of vegetables on dumpsites, irrigation with contaminated water, and application of animal waste to enrich the soil are among the numerous factors that could enhance the isolation of bacteria and fungi from vegetables (Arienzo *et al*., 2020).

The mean bacterial load of ready to eat vegetables sold in Kano revealed that all the samples collected had varying level of bacterial contamination ranging from 1.32×10^{5} CFU/ml to 1.45×10^{6} CFU/ml (Table 1), which is in the range of values reported by Feroz *et al*. (2016) in their study on vegetables in Bangladesh but at variance with the report of Amoah *et al*. (2006) in Ghana. It was observed that lettuce samples collected from Yankaba Kasua recorded the highest bacterial load and cucumber sample obtained from Kasua turawa on the other hand recorded the lowest mean bacterial load. The high mean bacterial load may suggest improper handling of vegetables from harvest through transportation and handling within the market space (Mritunjay and Kumar, 2017). Aerobic colony count is an indicator of food quality and potential shelf life, it does not relate to food poisoning and infections (Buyukunal *et al*., 2015). There was a significant difference (P-value= 0.038) between the mean bacterial loads of samples collected from both markets. Despite the relatively high mean bacterial load, none of the ready to eat vegetables shows any evidence of spoilage.

Fungi contamination is one of the safety challenges of ready to eat vegetables. The mean fungal count of ready to eat vegetable depicted in Table 2 shows that the highest mean fungal count was observed in cabbage samples obtained from Yankaba Kasua (8.8×10^{3} MC/ml) and carrot from Kasua Turawa (8.0×10^{3} MC/ml) whereas the least fungal count was observed in lettuce sample obtained from Kasua turawa (3.2x10^3 MC/ml). The mean fungal count in this study is at variance with the study of Gómez-Govea *et al*. (2012) where they recorded a higher fungal load from fruits and vegetables in Mexico.

In this study, various bacterial species were isolated in all the samples collected, similar to the study of Mritunjay and Kumar (2017). The frequency of bacterial isolates from ready to eat vegetables depicted in Table 3 shows that *Escherichia coli* and *Staphylococcus aureus* were the predominant isolates accounting for 28.75% and 23.75% respectively. There was a higher recovery of *E. coli* from lettuce samples (30.43%), similar to the report of Halablab *et al*. (2011). *E. coli* is a gram negative bacteria which is an indicator of faecal contamination of food which abound in our environment. It was argued by Mritunjay and Kumar (2017) that intrinsic adherence properties of gram negative bacteria to the surfaces of vegetables may help them survive washing and sanitizing steps during their preparation. The frequency of isolation of *S. aureus* in this study is in agreement with 23% *S. aureus* recovery from ready to eat vegetables in Brazil (Baraquet *et al*., 2021) but at variance with the 41.5% *S. aureus* recovery from fresh cut vegetables sampled by Faour-Klingbeil *et al*. (2016). *S. aureus* is ubiquitous in human body as part of the normal flora of the anterior nares, nasopharynx and the skin (Forbes *et al*., 2007) which may in-turn contaminate vegetables through the hand of the handlers. Conversely, Shigella species and *Enterobacter* species were the least bacterial specie isolated and identified in this study. Interestingly, no *Salmonella* species was isolated from this study, similar to the study of Amaoh (2014). This finding is contrary to the submission of Buyukunal *et al*. (2015) who reported 3.83% *Salmonella* species from the ready to eat vegetables assayed in their study. According to European Regulation (EC) No 1441/2007, the absence of *Salmonella* species is considered as an essential criterion to define the safety of ready to eat vegetables (Arienzo *et al*., 2020). Leafy vegetables are a group with a very high microbiological risk due to the soil for cultivation and cross contamination from the handlers (Feroz *et al*., 2020). The trend of bacteria isolated from this study is similar to what was reported by Yafetto *et al*. (2019) in their study.

The frequency of fungi identified in the ready to eat vegetables assayed in this study shows that member of the *Aspergillus* genera were found to be the most common fungi specie identified in all the samples accounting for 58.46% of the fungi identified (Table 4). The least fungi identified was found to be *Geotrichum* species identified in cabbage and cucumber samples obtained from Yankaba and Kasua Turawa markets respectively.

The lack of stringent decontamination procedures for the vans transporting vegetables, non-usage of cooling vans for transporting vegetables and display of vegetables under hot sun might be responsible for the proliferation of microorganisms in ready to eat vegetables.

Table 1: Mean Bacterial Load (10^4) of Ready to Eat Vegetable

Vegetable	N	Yankaba Kasua (10^4 CFU/ml)	Kasua Turawa (10^4 CFU/ml)	Total (10^4 CFU/ml)	*P*-Value
Cabbage	10	134 ± 8.45	105 ± 11.08	119.7 ±17.71	
Carrot	10	51.2 ± 31.4	37 ± 35.92	44.11 ±32.68	0.038
Cucumber	10	17.8 ± 6.45	13.2 ± 3.7	15.5 ±5.52	
Lettuce	10	145 ± 24.39	129 ± 9.55	137.4 ±19.3	
Total	40	87.05±58.54	71.3 ±52.13	79.18 ±55.29	

CFU/ml- colony forming unit per ml

Table 2: Fungi Load (10^3) of Ready to Eat Vegetables Sold in Kano

Vegetable	N	Yankaba Kasua (10^3 MC/ml)	Kasua Turawa (10^3 MC/ml)	Total (10^3 MC/ml)	*P*-Value
Cabbage	10	8.8 ± 2.59	7.8 ± 3.70	8.3 ± 3.06	
Carrot	10	4.6 ± 2.70	8.0 ± 3.67	6.3 ± 3.53	0.220
Cucumber	10	3.4 ± 1.14	6.0 ± 2.44	4.7 ± 2.26	
Lettuce	10	4.4 ± 2.07	3.2 ± 1.30	3.8 ± 1.75	
Total	40	5.3 ± 2.94	6.25± 3.35	5.8 ± 3.15	

MC/ml- mould count per ml

Table 3: Frequency of Bacteria Isolated from Ready to Eat Vegetables

Sample	**Cabbage**		**Carrot**		**Cucumber**		**Lettuce**		
Bacteria	Y N (%)	T N (%)	Y N (%)	T N (%)	Y N (%)	T N (%)	Y N (%)	T N (%)	Total N (%)
Escherichia coli	4(19.04)	2(9.52)	3(17.65)	1(5.88)	3(15.79)	3(15.79)	5(21.74)	2(9.00)	23(28.75)
Staphylococcus aureus	3(14.29)	3(14.29)	3(17.65)	1(5.88)	3(15.79)	1(5.26)	2(9.00)	3(13.04)	19(23.75)
Listeria monocytogenes	0(0.0)	1(4.76)	0(0.0)	0(0.0)	2(10.53)	0(0.0)	0(0.0)	1(4.35)	4(5.00)
Shigella specie	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.35)	0(0.0)	1(1.25)
Klebsiella pneumoniae	2(9.52)	3(14.29)	1(5.88)	1(5.88)	1(5.26)	1(5.26)	1(4.35)	1(4.35)	11(13.75)
Pseudomonas aeruginosa	1(4.76)	1(4.76)	1(5.88)	1(5.88)	0(0.0)	1(5.26)	1(4.35)	0(0.0)	6(7.5)
Enterobacter specie	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.26)	0(0.0)	0(0.0)	1(1.25)
Bacillus Specie	0(0.0)	0(0.0)	2(11.76)	3(17.65)	1(5.26)	2(10.53)	2(9.00)	2(9.00)	12(15.00)
Citrobacter freundii	1(4.76)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.35)	1(4.35)	3(3.75)
Total	21(100.0)		17(100.0)		19(100.0)		23(100.0)		80(100.0)

Y- Yankaba Kasua T-Kasua Turawa

Table 4: Frequency of Fungi Identified in Ready to Eat Vegetables

Sample	**Cabbage**		**Carrot**		**Cucumber**		**Lettuce**		
Fungi	**Y N (%)**	**T N (%)**	**Y N (%)**	**T N (%)**	**Y N (%)**	**T N (%)**	**Y N (%)**	**T N (%)**	**Total N (%)**
Aspergillus niger	3(16.67)	3(16.67)	1(7.69)	5(38.46)	4(23.53)	4(23.53)	4(23.53)	4(23.53)	28(43.08)
Aspergillus fumigatus	2(11.11)	1(5.56)	2(15.38)	1(7.69)	1(5.88)	1(5.88)	1(5.88)	1(5.88)	9(13.85)
Fusarium species	1(5.56)	1(5.56)	0(0.0)	0(0.0)	1(5.88)	2(11.76)	1(5.88)	1(5.88)	7(10.77)
Penicillium species	2(11.11)	2(11.11)	1(7.69)	1(7.69)	3(17.64)	0(0.0)	2(11.76)	1(5.88)	12(18.46)
Rhizopus specie	2(11.11)	0(0.0)	2(15.38)	0(0.0)	1(5.88)	0(0.0)	0(0.0)	1(5.88)	6(9.23)
Aspergillus species	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.88)	0(0.0)	1(1.54)
Geotrichum species	1(5.56)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.88)	0(0.0)	0(0.0)	2(3.08)
Total	18(100.0)		13(100.0)		17(100.0)		17(100.0)		65

Y- Yankaba Kasua T-Kasua Turawa

CONCLUSION

Results generated from this study have clearly shown that ready to eat vegetables harboured high microbial load majorly *E. coli*, *S. aureus* and *Aspergillus* species were isolated which could pose a potential health hazard to consumers. The finding suggest that food safety practices along the value chain are not good enough and therefore necessitate the need for awareness on critical areas that would improve the safety of ready to eat vegetables being sold to consumers. The results obtained equally highlights the need for good microbiological practice in the handling of ready to eat vegetables, hence the need for application of critical control point of washing with brine and vinegar during preparation. Good hygiene practices must be implemented from the farm, during transportation and when displaying in the market.

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