

Microbial Hazards and Public Health Risks Associated with Non-Refrigerated Ready-to-Eat Vegetable Salads

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Abstract

This study investigates the microbial quality of ready-to-eat (RTE) vegetable salads sold in Eke-Awka market, Anambra State, Nigeria. Five salad samples were collected and analyzed for total bacterial and fungal counts using Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA), and Sabouraud Dextrose Agar (SDA). Bacterial isolates were identified through Gram staining and biochemical tests, while fungal isolates were characterized using macroscopic and microscopic techniques. The results revealed significant variations in microbial loads among the samples. Total bacterial counts ranged from 9.0×10^4 to 1.75×10^5 CFU/g on NA, with all samples recording TNTC (Too Numerous to Count) on EMB, 3.0×10^4 to 9.0×10^4 CFU/g on MSA. Fungal contamination was highest in Sample A (4.1×10^2 CFU/g) and lowest in Sample C (1.7×10^2 CFU/g). The predominant bacterial isolates included *Staphylococcus* spp., *Escherichia coli*, and *Proteus* spp., while fungal isolates identified were *Aspergillus niger* (60%) and *Rhizopus* spp. (40%). This study reveals the potential risks of microbial contamination in non-refrigerated RTE vegetable salads, emphasizing the need for improved hygiene practices during harvesting, processing, and vending to ensure consumer safety. Enhanced public awareness and strict adherence to food safety regulations are recommended to mitigate these risks.

Keywords: Ready-to-Eat Vegetables Salads, Microorganisms, Microbial Contamination, Food Safety

Introduction

Salads have a rich and diverse history that spans thousands of years; when people consumed raw vegetables, fruits and herbs [1]. The early 20th century saw the emergence of pre-packaged salads and salad dressings, increasing convenience and accessibility [2]. Today, salads continue to adapt to changing tastes and dietary needs, with plant-based and vegan options on the rise with increased emphasis on sustainability and eco-friendliness [3]. Salads are fresh vegetables which require minimal washing and processing and cut into desired shapes and sizes with knives and other shredding utensils and usually served alongside other foods including rice [4]. These salads typically consist of a mixture of raw or lightly cooked vegetables, fruits, and sometimes proteins, packaged and sold as a complete meal [5]. However, the increasing demand for ready-to-eat vegetable salads has raised concerns about food safety and public health [6].

The lack of refrigeration during storage, transportation, and displays of ready-to-eat vegetable salads creates an environment conducive for bacterial growth, posing a significant risk to consumer health. Vegetable salads can be contaminated with a wide range of microorganisms, including pathogenic bacteria and fungi, during preparation, handling, and storage. If not managed properly, these microorganisms can multiply rapidly, leading to foodborne illnesses [7]. The prevalence of these microbial contaminants can vary depending on factors such as: the type of vegetables used, handling practices, temperature, hygiene practices during preparation and geographic location [8-10]. It is essential to note that even if the vegetables appear fresh and visually appealing, they can still harbour microbial contaminants and so, these factors should be kept in mind in order to minimize the risk of microbial bacterial contamination [11-14].

If ingested, they can cause foodborne illnesses, ranging from mild gastroenteritis to life-threatening conditions. Microbial adulteration can also occur during any of the steps in the farm-to-consumer continuum (production, harvest, fresh-cut processing, wholesale storage, transportation or retailing and handling) and this contamination can arise from environmental, animal or human sources [15]. Raw vegetables regularly get contaminated with pathogenic microorganisms during harvesting, post harvesting handling, processing, and distribution processes. This highlights the need for targeted interventions and education programs to ensure food safety practices are implemented across all levels of production [16, 13].

The lack of standardized guidelines for handling and storing ready to eat vegetable salads also contributes to the risk of contamination [17, 18]. Despite these potential health risks, there is limited research on the types and prevalence of bacterial and fungal isolates associated with non-refrigerated ready to eat vegetable salads. This knowledge gap necessitates an investigation into the microbial contaminants present in these foods and explore their microbiological safety. This study investigates the bacterial and fungal isolates associated with non-refrigerated ready to eat vegetable-salads, with a focus on identifying the types and prevalence of bacterial contaminants.

Materials and Methods

Sample Collection

Five ready-to-eat vegetable salad samples were obtained from different street food vendors at Eke-Awka market in Anambra State. The Samples were placed in clean plastic plates, labelled and promptly transported to the microbiological laboratory for laboratory analysis.

Sample Analysis

One gram (1g) of the vegetable salad samples were weighed and aseptically introduced into 10ml of sterile peptone water, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using peptone water as the diluents. 1 ml of appropriate dilutions (10⁻³) of each sample was pour plated in sterile plates of Nutrient agar (NA), Eosin Methylene Blue (EMB), Mannitol Salt Agar (MSA), and Sabouraud Dextrose Agar (SDA). The culture plates were incubated at 37°C aerobically for 24-48hours. Plates were examined after 24 hours of incubation, and colonies of organisms observed were sub cultured on freshly prepared agar plates. Developing colonies on all plates were counted to obtain total viable.

Characterization and Identification of the Isolates

The identification of the bacterial and fungal isolates was based on both microscopic and macroscopic features [19]. The characterization of the bacterial isolates was performed, by employing Gram staining reaction and biochemical tests, while the fungal isolates were characterized through macroscopic examination and staining with lactophenol cotton blue, as described by Bergey's Manual of Determinative Bacteriology, 9th edition (1994).

Gram Reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds.

Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (×100). Purple colour indicated Gram-positive organisms while red or pink colour indicated Gram-negative organisms.

Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

Simmons Citrate Test

A 24h old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

Catalase Test

Exactly 3ml of 3% solution of hydrogen peroxide (H₂O₂) was transferred into a sterile test tube. Then, 3 loopful of a 24 hr pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

Indole Test

A loopful of an 18-24 hr culture was used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation was done at 35–37 °C first for 24 hours and further for up to 48 hours. Test for indole was done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

Methyl Red Test

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl Red–Voges Proskauer (MR–VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

Voges Proskauer (Vp) Test

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR–VP broth was aseptically transferred into a sterile test tube. Then 0.6ml of 5% α-naphthol was added, followed by 0.2ml of 40% KOH (It was essential that these reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand un-

disturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

Urease Test

A heavy inoculum from an 18- to 24-h pure culture was used to streak the entire surface of the Christensen's Urea Agar slant. Care was taken not to stab the butt of the slant, as it served as a color control. The inoculated tubes were incubated with loosed caps at a temperature of 35°C. The slants were observed for a color change at various time intervals, including 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by the appearance of a bright pink (fuchsia) color on the slant, which might extend into the butt of the slant.

Carbohydrate Test

In the carbohydrate utilization test, basal medium containing single carbohydrates, bromo-cresol purple, bromo-thymol blue, Rade's blue or phenol red is added to the medium as pH indicators. A small inverted tube called Durham's tube is also immersed into the medium to test for gas production. Phenol red carbohydrate broth was dispersed into test tubes and sterilized. The organisms were inoculated into the test tubes and incubated for 18-24h at 35°C-37°C and observed for colour change from red to yellow. Acid production is detected by colour change, while gas displaces the media inside the tube producing visible air bubble.

Phenol Oxidase Detection Test

Phenol oxidase detection test was conducted using Niger seed agar (*Guizotia abyssinica*). Most isolates of *Cryptococcus neoformans* produces phenol oxidase. *Cryptococcus neoformans* is the only specie that produces 3,4- dihydroxyl, phenyl, alanine and an iron compound known as ferric citrate. *Cryptococcus neoformans* oxidizes O-diphenol to melanin which produces a brown to black color. The incubation of this process is done for 7days at 25°C, then observe for brown to black colonies.

Isolation and Characterization of the Fungi

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the modified slide culture technique using lactophenol cotton blue stain for the microscopic evaluation under X10 and X40 magnification of the microscope with reference to the Manual of Fungal Atlases [20].

Germ Tube Tests

A yeast colony from the culture plate was inoculated into human serum and incubated at 35–37°C for 2 hours. Sprouting tube-like cells were observed under the microscope at a magnification of x100.

Corn-Meal Agar Morphology Test

The test organisms were inoculated in the corn meal agar containing 1% Tween 80 and trypan blue, and incubated at 30°C for 48h. Microscopic morphological features on corn-meal agar differentiate the genera *Cryptococcus*, *Sacharomyces*, *Trichosporium*, *Candida* and *Geotricum*.

Morphological Characterization

The morphological features of fungal colonies were evaluated based on the criteria outlined in Atlas of Clinical Fungi. The Fungi were characterized by their shape, spore characteristics, color or pigmentation, and size.

Result

The total bacterial count (CFU/g) of the five ready-to-eat vegetable salad samples on three different media; Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), and Mannitol Salt Agar (MSA), is summarized in Table 1. Among the samples, Sample E exhibited the highest bacterial load across all media, with a total count of 1.28×10^5 CFU/g on NA, TNTC (Too Numerous to Count) on EMB, and 9.0×10^4 CFU/g on MSA. Sample B also recorded a high count on NA, reaching 1.75×10^5 CFU/g, while its count on MSA was comparatively lower at 4.6×10^4 CFU/g. Sample C recorded the lowest bacterial load on MSA (3.0×10^4 CFU/g) but had a moderate count on NA (9.9×10^4 CFU/g). Across all samples, bacterial counts on EMB were consistently reported as TNTC.

Table 1: Total Bacterial Count (CFU/g)

SAMPLES	Total Bacterial Count (CFUg-1) on NA	Total Bacterial Count (CFUg-1) on EMB	Total Bacterial Count (CFUg-1) on MSA
A	9.0×10^4	TNTC	7.3×10^4
B	1.75×10^5	TNTC	4.6×10^4
C	9.9×10^4	TNTC	3.0×10^4
D	9.7×10^4	TNTC	6.0×10^4
E	1.28×10^5	TNTC	9.0×10^4

Key

- NA- Nutrient agar
- EMB- Eosin Methylene Blue agar
- MSA- Mannitol Salt agar
- TNTC- Too numerous to count

Table 2 shows the morphological and biochemical identifications of the various bacterial isolates, isolated from the five samples of ready-to-eat vegetable salads. The bacteria isolates were identified and confirmed as *Staphylococcus* spp., *Escherichia coli*, and *Proteus* spp.

Table 2: Morphological and Biochemical Identifications of the Various Bacterial Isolates.

Isolate	Size	Colour	Texture	Elevation	Form	Margin	Gram	Glu	Fru	Lac	Mal	Cit	Cat	Ind	MR	VP	Identity
A	Moderate	Pale yellow	Mucoid	Flat	Irregular	Lobate	+cocci	+	+	+	+	-	+	-	+	+	Staphylococcus spp.
B	Large	White	Mucoid	Raised	Irregular	Undulate	-rod	+	+	+	+	+	+	-	-	+	Proteus spp.
C	Large	Pale pink	Mucoid	Flat	Irregular	Undulate	-rod	+	+	+	+	-	+	-	+	-	Escherichia coli

Key

Glu- Glucose; Fru- Fructose; Lac- Lactose; Mal- Maltose; Cit- Citrate test; Cat- Catalase test; Ind- Indole test; MR- Methyl Red test; VP- Voges Proskauer test

The total fungal count (CFU/g) and the number of fungal colonies observed on Sabouraud Dextrose Agar (SDA) for the five ready-to-eat vegetable salad samples are presented in Table 2. Sample A exhibited the highest fungal contamination, with a total fungal count of 4.1×10^2 CFU/g, while Sample C recorded the lowest fungal load among all the samples, with a total fungal count of 1.7×10^2 CFU/g.

Table 3: Total Fungal Count (CFU/g)

SAMPLES	No. of Fungal colonies on SDA	Total Fungal Count (CFUg-1) on SDA
A	41	4.1×10^2
B	26	2.6×10^2
C	17	1.7×10^2
D	24	2.4×10^2
E	22	2.2×10^2

Key

SDA - Sabouraud dextrose agar

Table 4 shows the microscopic and macroscopic characteristics of fungal isolates on the ready-to-eat vegetable salad samples. This table provides evidence for the occurrence of fungal isolates. The fungal isolates were identified and confirmed as *Rhizopus* spp. and *Aspergillus niger*.

Table 4: Microscopic and Macroscopic Characteristics of Fungal Isolates from the Vegetable Salad Samples

Isolates	Microscopic characteristics	Macroscopic characteristics	Probable Organism
1	round with flattened bases, black sporangia at the tips of the sporangiophores are rounded and produce numerous nonmotile multinucleate spores	Cotton-candy like with initially white colony and turns grey to yellowish brown in time	<i>Rhizopus</i> spp.
2	Septate, hyaline hyphae and long, smooth conidiophores with large, spherical vesicles.	Dense, black colony with reverse colour of pale yellow. Cottony texture that became granular as the colonies mature.	<i>Aspergillus niger</i>

Table 5 shows the occurrence of the various fungal isolates on the ready-to-eat vegetable salad samples. The result showed that *Aspergillus niger* have the highest occurrence of 60%, while *Rhizopus* spp. had a lower occurrence of 40%.

Table 5: Frequency and Proportions of the Identified Organisms

Organisms	Proportions	Frequency in percentage
<i>Rhizopus</i> spp.	0.4	40%
<i>Aspergillus niger</i>	0.6	60%

Discussion

Vegetables and fruits, like other living organisms, possess a natural microbial flora. However, additional contaminants may be introduced during harvesting, transportation, and processing for consumption (Ofor et al. 2022). This study evaluated the total bacterial and fungal counts in ready-to-eat salads to assess

their microbiological quality and potential public health risks. Although the total viable count is not a legislative criterion for ready-to-eat salads, it serves as an important indicator of hygienic and sensory quality, providing valuable insights into the overall microbiological status of the food. The findings from this study revealed variations in the total bacterial and fungal counts

across five different vending points within Awka metropolis. This variation in microbial load among street-vended vegetable salads is expected, as each vending site has unique features and activities that likely influence the bacterial contamination levels of ready-to-eat (RTE) food items sold there. The open and exposed nature of these vending sites further contributes to the potential for microbial contamination.

The mean bacterial load of RTE vegetable samples showed varying levels of contamination, ranging from 9.0×10^4 to 1.75×10^5 CFU/g on nutrient agar (NA), 3.0×10^4 to 9.0×10^4 CFU/g on mannitol salt agar (MSA), and "too numerous to count" (TNTC) on eosin methylene blue agar (EMB) (Table 1). These values are higher than those reported by Ajiboye and Emmanuel (2021) for RTE vegetables in Ilorin, but lower than the findings of Saka et al. (2022) in their study on RTE vegetables sold in Yankaba Market, Kano, and Adeyemi et al. (2019) for fresh vegetable salads from vendors in Oyo metropolis. The high mean bacterial load observed in this study may indicate improper handling practices during harvesting, transportation, and retailing, as noted by Mritunjay and Kumar (2017). However, despite the relatively high bacterial counts, none of the RTE vegetable samples showed visible signs of spoilage.

The bacterial strains isolated in this study were identified through morphological and biochemical tests as *Staphylococcus* spp., *Escherichia coli* (*E. coli*), and *Proteus* spp. *Staphylococcus* spp. are abundant in water and soil, including canal environments [21]. Previous studies, such as those by Kayombo and Mayo (2018) and Ullah et al. (2012), have highlighted the use of polluted river water or wastewater by urban farmers for irrigating vegetable crops, increasing the risk of microbial contamination. Vegetables grown under such unsanitary conditions are highly susceptible to contamination [22]. *Staphylococcus* spp. have been linked to a wide range of infections in humans and animals [23]. These bacteria can produce toxins in food, causing foodborne illnesses like food poisoning [24]. Similarly, *E. coli* is a frequent cause of foodborne disease outbreaks, often indicating fecal contamination and poor hygienic practices among food handlers [25]. Certain *E. coli* strains can cause gastroenteritis and diarrhea, particularly in children from developing countries [26, 27]. *Proteus* spp., as reported by Ulfat et al. (2022), are widespread in the environment, including animals, soil, and polluted water, and are part of the normal flora of the human gastrointestinal tract. Several studies have reported the prevalence of pathogenic microbes in fruits and vegetables.

Moses et al. (2016) detected *E. coli* O157 in fruits and vegetables from farms in Akwa Ibom State, Nigeria, while Ehimemen et al. (2019) isolated *Staphylococcus aureus* and *E. coli* from produce sold in North-Western Nigeria. Wadamori et al. (2017) identified *Salmonella* spp., *E. coli* O157:H7, *Staphylococcus aureus*, *Campylobacter* spp., and *Listeria monocytogenes* as common pathogens contaminating fresh produce. Similarly, Sabbithi et al. (2014) reported *Salmonella* spp., *Staphylococcus aureus*, and *Yersinia* at unacceptable levels in salads served with street foods in Hyderabad. Interestingly, no *Salmonella* species were isolated in this study, aligning with findings by Amaoh (2014). According to European Regulation (EC) No 1441/2007, the absence of *Salmonella* species is an essential criterion for defining the safety of ready-to-eat vegetables [28].

Fungal contamination poses a significant safety challenge for ready-to-eat (RTE) vegetables. The mean fungal counts for RTE vegetable samples, as depicted in Table 3, revealed the highest fungal load in Sample A (4.1×10^2 CFU/g) and the lowest in Sample C (1.7×10^2 CFU/g). These findings indicate a lower fungal count compared to the results reported by Adeyemi et al. (2019), who observed higher fungal loads in RTE vegetable salads sold in Oyo. The fungi identified in this study, including *Rhizopus* spp. and *Aspergillus niger*, are consistent with previous findings from RTE foods in Gombe (Ibrahim et al., 2022), RTE vegetable salads in Oyo and RTE vegetables sold in Kano [29, 30]. Among the fungi identified, *A. niger* was the most frequently isolated species, accounting for 60% of the total fungi identified across all samples (Table 5) [31-35].

Conclusion

This study assessed the microbiological quality of ready-to-eat (RTE) vegetable salads sold at five vending points in Awka metropolis. The results revealed significant variations in bacterial and fungal contamination levels, reflecting the impact of environmental conditions and handling practices at these locations [36-40]. This study shows the urgent need for improved hygiene practices across the RTE vegetable supply chain, from cultivation to vending, to minimize microbial contamination and ensure consumer safety. Continuous monitoring, public awareness campaigns, and adherence to food safety standards are essential to safeguarding public health [41-44].

References

- Holt, D. (2020). Historical Perspective on Food and Health. *Journal of Culinary Medicine*, 2(2), 15-22.
- Smith, A. F. (2020). The Evolution of the American Salad. *Journal of Food Studies*, 10(1), 01-12.
- Jones, A., Smith, P. and Brown, L. (2023). Evolving Salads: Plant-Based, Vegan and Sustainable Trends. *Journal of Food Science and Technology*, 60(3), 1241-1250.
- Ababio, P. F., Lovatt, P. (2014). Consumer perceptions of food safety in Ghana. *Food Control*, 40, 324-331.
- United States Department of Agriculture (USDA). (2020). Nutritional guidelines for fresh produce. Retrieved from <https://www.usda.gov/>.
- World Health Organization (WHO). (2022). Microbial risks in ready-to-eat foods. Retrieved from <https://www.who.int/foodsafety>.
- Centers for Disease Control and Prevention (CDC). (2020). Foodborne illness and germs. Retrieved from <https://www.cdc.gov/foodsafety/>.
- Okoli, F. A., Okonkwo, N. N., Agu, K. C., Nwobu, W. C., Uwanta, L. I., Ifediegwu, M. C., & Umeoduagu, N. D. (2023). Identification of potential microbial contaminants from stored pap. *International Journal of Progressive Research in Engineering Management and Science*, 3(9), 409-415.
- Victor-Aduoju, A.T., Okonkwo, N.N., Okoli, F.A., Agu, K.C., Okoye, C.W., Awari, V.G., ... & Umeoduagu, N.D. (2023). Comparative Analysis of microbial Load of Water in Selected Hostels in Ifite, Awka. *International Journal of Progressive Research in Engineering Management and Science*, 3(9), 400-408

10. Ezenwelu, C. O., Duruamaku, P. U., Udemezue, O. I., Agu, K. C., Oparaji, E. H. (2024). Chemical Composition and Microbial Quality Assessment of Conventional Yoghurts within Awka Metropolis. *Asian Journal of Research in Biology*, 7(1), 25-35.
11. Agu, K. C., Orji, M. U., Onuorah, S. C., Ejurefa, S. O., Anaukwu, C. G., Okafor, U. C., ... & Anyaegbunam, B. C. (2014). Comparative Bacteriological Analysis of Ready-to-Eat Vegetables Salad Sold by Various Food Vendors in Awka. *American Journal of Life Science Researches*, 2(4), 458-465.
12. Anaukwu, C. G., Ugwuoke, G. O., Ikech, O. C. O., Agu, K. C. (2015). Preliminary study of bacterial isolates from indigenous ready-to-eat salad vegetables. *American Journal of Life Science Researches*, 3(4), 282-286.
13. World Health Organization (WHO). (2018). Food safety: Ensuring global public health. Retrieved from <https://www.who.int/foodsafety>.
14. Awari, V. G., Umeoduagu, N. D., Agu, K. C., Okonkwo, N. N., Ozuah, C. L. Victor-Aduloju, A. T. (2023). The Ubiquity, Importance and Harmful Effects of Microorganisms: An Environmental and Public Health Perspective. *International Journal of Progressive Research in Engineering Management and Science*, 3(12), 1-10.
15. María, J. G., Hernández, M. E., Rivera, S. L. (2017). Microbial contamination in fresh-cut vegetables: Risks and prevention strategies. *Journal of Food Microbiology*, 24(5), 232-239.
16. Mbachu, A. E., Etok, C. A., Agu, K. C., Okafor, O. I., Awah, N. S., Chidi-Onuorah, L. C., ... & Ikele, M. O. (2014). Microbial quality of kunu drink sold in Calabar, Cross River State, Nigeria. *Journal of Global Biosciences*, 3(2), 511-515.
17. Food and Drug Administration (FDA). (2019). Food safety modernization act (FSMA). Retrieved from <https://www.fda.gov/food>.
18. Obianom, O. A., Ogbonna, U. S. A., Agu, K. C., Ozuah, C. L., Okonkwo, N. N., Victor-Aduloju, A. T., Umeoduagu, N. D. (2023). Microbiological and Phytochemical Evaluation of Jackfruit and Soursop. *International Journal of Science Academic Research*, 11(4), 6662-6674.
19. Cheesbrough, M. (2006). District laboratory practice in tropical countries, (2nd ed.). Cambridge university press.
20. Agu, K. C., Chidozie, C. P. (2021). An Improved Slide Culture Technique for the Microscopic Identification of Fungal Species. *International Journal of Trend in Scientific Research and Development*, 6 (1), 243-254. URL: www.ijtsrd.com/papers/ijtsrd45058.pdf.
21. Ulfat, M., Abad, Z., Ali, N., Sarwar, S., Jabeen, K., Abrar, A. (2022). Screening, biochemical characterization, and antibiotic resistance/susceptibility of bacteria isolated from native soil and water samples. *Brazilian Journal of Biology*, 84, e254016.
22. Woldetsadik, D., Drechsel, P., Keraita, B., Itanna, F., Erko, B., Gebrekidan, H. (2017). Microbiological quality of lettuce (*Lactuca sativa*) irrigated with wastewater in Addis Ababa, Ethiopia and effect of green salads washing methods. *International Journal of Food Contamination*, 4(3), 1-9.
23. Madigan, M. T., Martinko, J. (2005). Brock biology of microorganisms, 11th edn. Upper Saddle River, NJ: Prentice Hall.
24. Cenci-Goga, B. T., Karama, M., Rossitto, P. V., Morgante, R. A., Cullor, J. S. (2003). Enterotoxin production by *Staphylococcus aureus* isolated from mastitic cows. *Journal of food protection*, 66(9), 1693-1696.
25. Bakobie, N., Addae, A. S., Duwiewuah, A. B., Cobbina, S. J., Miniyila, S. (2017). Microbial profile of common spices and spice blends used in Tamale, Ghana. *International journal of food contamination*, 4(10), 1-5.
26. Akter, N. (2016). Study on bacteriological quality of street-vended foods collected from different private universities in Dhaka City, Bangladesh (Unpublished bachelor's thesis). East West University, Dhaka, Bangladesh.
27. Adams, M. R., Moss, M. O. (2008). Bacterial agents of foodborne illness. Cambridge, UK: The Royal Society of Chemistry. In *Food Microbiology* (3rd ed., pp. 182-268).
28. Arienzo, A., Murgia, L., Fraudentali, I., Gallo, V., Angelini, R., Antonini, G. (2020). Microbiological quality of ready-to-eat leafy green salads during shelf-life and home-refrigeration. *Foods*, 9(10), 1421.
29. Adeyemi, O. A., Fejukui, B. M., Adeyemi, O. O. (2019). Microbial contamination of fresh vegetable salads from food vendors in Oyo Metropolis. *Nigerian Journal of Pure and Applied Sciences*, 32(1), 3374-3379.
30. Sabbithi, A., Naveen Kumar, R., Kashinath, L., Bhaskar, V., Sudershan Rao, V. (2014). Microbiological quality of salads served along with street foods of Hyderabad, India. *International Journal of Microbiology*, 2014(1), 932191.
31. Ajiboye, A. E., Emmanuel, T. O. (2021). Assessment of bacterial contamination in ready-to-eat fruits and vegetables sold at Oja-Oba market, Ilorin, Nigeria. *African Journal of Biomedical Research*, 203-209.
32. Amoah, P., Drechsel, P., Abaidoo, R. C., Ntow, W. J. (2006). Pesticide and pathogen contamination of vegetables in Ghana's urban markets. *Archives of environmental contamination and toxicology*, 50(1), 1-6.
33. Bergey, D. H., Holt, J. G. (1994). Bergey's manual of determinative bacteriology (9th ed.). Lippincott Williams & Wilkins.
34. Helen Ehim, N., Fatima Muk, M., Salisu, N. (2019). Prevalence of bacterial loads on some fruits and vegetables sold in kaduna central market, Northwestern Nigeria. *Journal of Applied Sciences*, 19(1), 20-24.
35. Kayombo, M. C., Mayo, A. W. (2018). Assessment of microbial quality of vegetables irrigated with polluted waters in Dar es Salaam City, Tanzania. *Environ. Ecol. Res*, 6(4), 229-239.
36. Enweani-Nwokelo, I. B., Ogbonna, U. S. A., Okwu, G. I., & Enweani, O. O. (2022). Harnessing the Underexploited Plant Species for Economic Growth in Nigeria. In *Medical Biotechnology, Biopharmaceutics, Forensic Science and Bioinformatics* (pp. 359-382). CRC Press.
37. Moses, A. E., James, R. A., Ekanem, U. S. (2016). Prevalence of *Escherichia coli* O157 in fruits, vegetables and animal fecal waste used as manure in farms of some Communities of Akwa Ibom State-Nigeria. *Central African Journal of Public Health*, 2, 22-27.
38. Mritunjay, S. K., Kumar, V. (2017). A study on prevalence of microbial contamination on the surface of raw salad vegetables. *3 Biotech*, 7(13), 01-09.

39. Ofor, M. O., Okorie, V. C., Ibeawuchi, I. I., Ihejirika, G. O., Obilo, O. P., Dialoke, S. A. (2009). Microbial contaminants in fresh tomato wash water and food safety considerations in South-Eastern Nigeria. *Life Sci. J*, 1, 80-82.
40. Ogbonna U. S. A., Okoh F.N., Obianom, O.A., Agu K.C., Anazodo C.A., Chidubem-Nwachinemere N.O., ... & Nwosu J. C. (2024) Phytochemical Composition & Bacterial Isolates Associated with Moringa Oleifera Leaves/Seeds. *Research and Analysis Journals*, 7(8), 40-53.
41. Ogbonna U. S. A., Okoh F. N., Obianom, O.A., Agu K. C., Abana C. C., Anazodo C.A., ... & Nwosu J.C. (2024) Nutritional Composition and Fungi Isolates Associated with Moringa Oleifera Leaves and Seeds. *Research and Analysis Journals*, 7(8), 54-60.
42. Saka, H. K., Ahmad, F. U., Abubakar, A. A. (2022). Microbiological quality assessment of ready-to-eat vegetables sold in Yankaba market, Kano, Nigeria. *Dutse Journal of Pure and Applied Sciences*, 8(3b), 163-169.
43. Ullah, H., Khan, I., Ullah, I. (2012). Impact of sewage contaminated water on soil, vegetables, and underground water of peri-urban Peshawar, Pakistan. *Environmental monitoring and assessment*, 184, 6411-6421.
44. Wadamori, Y., Gooneratne, R., Hussain, M. A. (2017). Outbreaks and factors influencing microbiological contamination of fresh produce. *Journal of the Science of Food and Agriculture*, 97(5), 1396-1403.