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Proximate Compositions and Bacterial Quality Assessment of Ready-to-Eat Vegetable Salads Hawked in Kaduna Metropolis

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Abstract

Foodborne outbreaks caused by the consumption of contaminated vegetables represent a major public health problem worldwide, especially in developing countries like Nigeria. Assessment of bacteriological quality is necessary in ensuring circulation of uncontaminated products. Randomly total of 240 samples of ready-to-eat vegetable salads were purchased from hawkers in four major areas in Kaduna metropolis. The proximate composition of the salad samples were determined using standard protocols. Pour plate method was used to isolate bacteria from the vegetable samples. The results obtained showed variations in the percentage proximate compositions of the vegetables analyzed. The total aerobic bacterial counts ranged from 0.61±1.29 to 7.56±2.88CFU/g. The bacteria isolated and identified were Escherichia coli (12.92%) and Salmonella sp. (2.08%). The presence of these bacteria isolates, which are capable of causing foodborne diseases associated with the consumption of contaminated vegetables, raises more concern over public health risk. Proper washing, handling and serving of the ready-to-eat vegetable salads will reduce the risk of foodborne diseases associated with bacteria.

keywords: vegetable salads, Aerobic bacterial count, proximate

INTRODUCTION

Salad is a mixture of fresh vegetables (tomatoes, cucumber, cabbage, Lettuce, Onion and Spices.) that provides rich sources of vitamins, minerals and dietary fibers of low fat and calories to the consumer. In recent years, salad has become a very popular component of the menu served at birthday and wedding parties; they are also sold in fast food centers in most major cities in Nigeria(Srinivasan *et al.*, 2022).The consumption rates of vegetables and vegetable salads have also greatly increased based on their proven medical and nutritional benefits. Recently, vegetables are sliced and beautifully arranged in layers in transparent plastic containers and hawked in almost every market, motor parks and other public places (Najib *et al.*, 2021). Media reports of unverified rampant cases of gastroenteritis following consumption of meals served with fresh vegetable salads have become a serious public health concern (Udo *et al.*, 2022). Salad has high water content because of its dressing, but it is low in calories and hence it is used by people who are aiming at weight loss, help in disorders and strokes. Moreover, the availability of potable water for proper washing of these vegetables is also lacking in different areas (Najib *et al.*, 2021). As a result of which dirty or contaminated water is used for washing which could lead to further increase in the microbial load because some people buy and eat such vegetables without further washing and also it can become contaminated with pathogenic microorganisms during harvesting, through human handling, harvesting equipment's and transport containers (Elexson *et al.*, 2017; New *et al.*, 2019). In view of the growing concerns on the bacteriological safety of the salads sold in Kaduna and increase in consumption of the salads by the teeming populace, there is need for the study on ready to eat vegetable salads hawked in Kaduna Metropolis.

MATERIALS AND METHODS

Study Area

This research was Carried out in four major areas within Kaduna metropolis, (Anguwan Rimi, Kawo, Rigasa and Kakuri) Kaduna State Nigeria. Kaduna State is located on the latitude 10.53 ^{0}N and longitude 7.44 ^{0}E with an elevation of 626m

above sea level. The state is in the north-west part of Nigeria.

Sample Size Determination

The sample size was determined using the following formula, (Cochran, 1977).

$N = [t^2 \times p (1 - p)]/m^2$

n = number of samples

m = margin of error= 0.05

p = percentage of existing prevalence=7.7% (Abakpa *et al.*,2015).

t = t-value at 95% Confidence Interval (CI) =1.96

Inputting the figures into the sample size formula,

$n= (1.96)^2 \times 0.077 \times (1- 0.077)/ (0.05)^2= 3.84 \times 0.077 \times 0.923/0.0025 =109$

Therefore, a proposed sample size of 240 was used in this study.

Collection of Sample

Total of 240 samples of ready to eat vegetable salads were obtained randomly from hawkers in four major areas in Kaduna metropolis, from Kaduna North, Kaduna south, kawo and Igabi. Forty (40) samples each of cabbage, lettuce, cucumber, tomato, onion and spices were collected. All samples collected from the sites were analyzed in the laboratory of the Department of Microbiology, Faculty of science, Kaduna State University.

Proximate Analysis of the vegetable salads components

The proximate composition of the vegetable salads components was analyzed according to the method described by Association of Office Analytical Chemist (AOAC ,2023); Adeniyi *et al.*, (2012) and Adeyeye (2018). The proximate parameters analyzed include moisture content, crude protein, ash content, crude fibre, crude fat and carbohydrates.

Determination of Total Mesophilic Aerobic Count.

The isolation of the total mesophilic count was conducted according to the methods adopted by FAO (2018). A stock solution was prepared aseptically by weighing 25g of the vegetables (cabbage, lettuce, cucumber, onion, tomato and spices) in a sterile conical flask using weighing balance and 225mL of sterile distilled water was added into the conical flask containing the 25g of the vegetables.From the mixture10-fold serial dilution was carried out as follows. Ten sterile test tubes were prepared each containing 9mL of distilled water. Serial dilution was made by transferring 1.0mL of the stock. Nutrient agar was prepared according to manufacturer's instruction and was allowed to cool to 45^0C.1.0mLof each of the dilutions was dispensed into sterile petri-plates and about 20.0mL of the sterile molten nutrient's agar was dispensed into the plates containing 1.0mL of aliquots. The plates were swirled gently on the bench and allowed to solidify. All the plates were incubated at 37^0C for 24h.The colonies were counted using a colony counter and calculated using the equation below

= (no of colonies x dilution factor)/ volume of inoculums

The dilution with the count of 30-300 CFU/g was used for subsequent analysis

Isolation of Coliforms

Most probable number method was used for detection of coliforms as 25g of the vegetable was weighed and placed in a sterile conical flask containing sterile 225mL of distilled water. Then three-fold serial dilutions were made.

Presumptive Test:

For the presumptive test,Ninetest tubes wereused with each containing an inverted durham tube with Lauryl Sulphate Tryptose (LST), 1.0 mL of the dilution was transferred into the test-tube in triplicate and all the test-tubes were incubated at 37^oC for 24 hours. All the positive tubes were used to carry out the confirmatory test.

Confirmatory test:

Test-tubes containing brilliant green lactose bile 2% (BGLB) with inverted Durham tube in the test tubes were prepared and one loopful of all the positive tubes was transferred and incubated at

37°C for 48 hours all the positive tubes were calculated from MPN tables.

Completed test

From the positive tubes obtained from the confirmatory test, a sterile wire loop was used to streak on EMB agar. Green metallic sheen was observed as a positive test.

Detection of *Salmonella* specie

Aseptically, 25g of the vegetable was weighed and added to a sterile conical flask containing 225mL of distilled water. The mixture was manually mixed vigorously by swirling the flask 25 times clockwise and 25 times anticlockwise. 1.0mL of the mixture was transferred to a 10mL selenite cysteine (SC) broth contained in a test tube and was mixed and incubated at 35 °C for 24h. A loopful of the mixture was streaked on xylose lysine deoxycholate (XLD) agar, and the plates were incubated at 35 °C for 24h. All the plates were examined for the presence of colonies (Cheesbrough, 2022).

Characterizations of Bacterial Isolates obtained.

The characterization of bacteria isolates from the vegetable salad components were based on Gram staining and biochemical tests which include urease, catalase, coagulase, indole, citrate, Methyl red (MR)test, Voges-proskauer(VP),Tripple sugar Iron Agar(TSI)test as described by Cheesbrough (2022).

RESULTS

The result of the proximate composition of cabbage, cucumber, lettuce, onion, spices and tomato is presented in Table 1. The presence of six constituents was determined, namely moisture content, ash, protein, crude fibre, lipid, and carbohydrate. A higher moisture content was found in tomato (20.89±0.05), while cabbage has the least moisture content of (14.94±0.05). However, a higher protein content was found in cabbage (12.72±0.05), while tomato has the least protein content of (10.83±0.07). Cucumber has the highest ash content of (10.20±0.6), while onion has the least ash content of (4.15±0.15). More also a higher crude fiber was found in lettuce (52.28±0.13), while cabbage has the least crude fiber content of (16.45±0.05). For lipid content spices has a higher content of (23.11±0.12) while onion has the least content of (0.56±0.06). Cucumber has a higher carbohydrate content of (43.35±0.06) compared to cabbage, onion, tomato, lettuce and spices while lettuce has the least carbohydrate content of (5.33±0.01).

The result of the mean aerobic mesophilic bacterial counts of cabbage, cucumber, lettuce, onion, spices and tomato is presented in Table 2. From the result samples collected in Kaduna south recorded highest mean bacterial count of 7.56±2.88 CFU/g in lettuce while samples collected in Igabi recorded least mean counts of 4.85±2.19 CFU/g. For cabbage and cucumber samples in Kaduna north has the highest mean bacterial counts of 4.19±1.63 CFU/g, 3.53±0.062 CFU/g, while samples collected in Igabi has the least mean bacterial counts of 2.0±2.16 CFU/g, 0.91±1.47 CFU/g. However, no growth was observed from the samples of onion and spices in all the locations. Highest mean counts of 1.91±2.04 CFU/g in tomato was found in Igabi while samples collected in Kaduna south has the least mean counts of 1.3±1.173 CFU/g. Furthermore, samples of lettuce have the highest mean bacterial counts in the four Local Governments of Kaduna State compared to the other samples analyzed. Similarly, samples collected in Igabi has the least mean bacterial counts in all the components of the salad except in samples of tomato in which samples from Igabi has the highest mean bacterial count.

The results of morphological characteristics, microscopy and biochemical identification of the isolates are presented in Table 3. The probable bacteria isolated were *Salmonella* species and *Escherichia coli*. The morphological characteristics of *Salmonella* species on salmonella shigella agar showed black mucoid colonies and for *Escherichia coli* on eiosine methylene blue agar showed green metallic sheen colonies. However, for microscopy, the isolates were Gram-negative bacilli. More also biochemical identification of suspected *Salmonella* species was urease -ve, catalase +ve, coagulase +ve, indole -ve, citrate -ve, MR +ve, Vp -ve, gas +ve, glucose +ve, lactose -ve, sucrose -ve and H_2S +ve. For the suspected *Escherichia coli*, it was urease -ve, catalase +ve, coagulase -ve, indole +, citrate -ve, MR +ve, VP -ve, gas +ve, glucose +ve, lactose +ve, sucrose +ve, and H_2S -ve.

The frequency of occurrence of the *E. coli* in ready-to-eat vegetable salad hawked in Kaduna metropolis is presented in Table 4. From the 240 samples, *E. coli* had a frequency of occurrence of 31(12.92%). Among the 40 samples of each of

the vegetable salad analyzed *E. coli* had the highest frequency of occurrence in lettuce 15(48.4%), followed by cabbage which had9(29.0%), furthermore cucumber had 4(12.9%) of *E. coli* and in tomato which had 3(9.7%) samples positive for *E. coli*. Lastly, *E. coli* was not found in all the samples collected from the onion and spices.

Table 1: Proximate compositions of cabbage, cucumber, lettuce, onion, spices and tomato

Sample	MC (%)	Protein (%)	ASH (%)	CF (%)	Lipid (%)	Cab.(%)
Cabbage	$14.94±0.05^{ab}$	$12.72±0.05^{bd}$	$8.05±0.05^{ca}$	$16.45±0.05^{ab}$	$7.56±0.05^{bc}$	$40.34±0.005^{ad}$
Cucumber	$16.97±0.005^{bc}$	$11.27±0.06^{ab}$	$10.20±0.6^{ab}$	$16.75±0.005^{dc}$	$1.72±0.02^{ad}$	$43.35±0.06^{ab}$
Lettuce	$15.10±0.04^{bd}$	$11.66±0.05^{ba}$	$10.22±0.22^{cb}$	$52.28±0.13^{cb}$	$5.92±0.02^{dc}$	$5.33±0.01^{ca}$
Onion	$17.69±0.03^{da}$	$11.52±0.04^{cb}$	$4.15±0.15^{aa}$	$23.38±0.08^{aa}$	$0.56±0.06^{aa}$	$43.07±0.01^{bd}$
Spices	$7.88±0.03^{cb}$	$11.40±0.01^{ba}$	$8.1±0.05^{ac}$	$40.46±0.04^{db}$	$23.11±0.12^{cd}$	$9.35±0.01^{da}$
Tomato	$20.89±0.05^{ab}$	$10.83±0.07^{aa}$	$8.05±0.05^{bc}$	$30.15±0.03^{ba}$	$4.38±0.08^{aa}$	$25.90±0.02^{da}$

Values are presented as mean ± SD, and values with different superscript within the same row indicates statistical significant difference (P<0.05). Key: MC: moisture content; CF: crude fibre; Cab: carbohydrate

Table 2: Mean Aerobic Mesophilic Bacterial counts of hawked ready to eat salad.

Location	Mean viable count ×10^2 (CFU/g)					
	Lettuce	Cabbage	Cucumber	Onion	Tomato	Spice	P-value
KN	$7.3±8.19^{ab}$	$4.19±1.63^{acdef}$	$3.53±0.062^{bcghi}$	$0.0±0.00^{dgjk}$	$1.3±1.173^{ehjl}$	$0.0±0.00^{fikl}$	<0.0001
KS	7.56±2.88	$3.61±3.96^{a}$	$1.31±1.70^{abcd}$	$0.0±0.00^{bef}$	$0.61±1.29^{ceg}$	$0.0±0.00^{dfg}$	<0.0001
KA	6.82±2.67	$3.01±3.46^{ab}$	$1.78±1.90^{acde}$	$0.0±0.00^{cfg}$	$0.94±1.52^{bdfh}$	$0.0±0.00^{egh}$	<0.0001
IG	4.85±2.19	$2.0±2.16^{abcd}$	$0.91±1.47^{aefg}$	$0.0±0.00^{be}$	$1.91±2.04^{cf}$	$0.0±0.00^{dg}$	<0.0001

Values are presented as Mean±Standard deviation p≤0.05. Values with the same superscript are non-significantly different. Key: KN-Kaduna north; KS- Kaduna south ; KA-Kawo; IG-Igabi; CFU/g- coliform forming unit/gram.

The frequency of occurrence of the *Salmonella* species in ready-to-eat vegetable salad hawked in Kaduna metropolis is presented in Table 5. From the 240 samples, *Salmonella* species had the frequency of occurrence of 5(2.08%). Among the 40 samples of each of the vegetable salad analyzed *Salmonella* species had the highest frequency of occurrence in lettuce 4(80%), followed by cabbage which had1(20.0%). Lastly, *Salmonella* species were not found in all the samples collected from cucumber, tomato, onion and spices.

Table 3: Cultural, Morphological and Biochemical Characteristics of suspected Bacterial isolates obtained from ready to eat vegetable salad hawked in Kaduna metropolis.

Characteristics	Probable Bacteria	
	Escherichia coli	*Salmonella* species
Gram reaction	-	-
Morphology	Rod	Rod
Urease	-	-
Catalase	+	+
Coagulase	-	-
Indole	+	-
Citrate	-	-
Methyle- Red	+	+
Vogas Proskauer	-	-
Gas	+	-
Glucose	+	+
Lactose	+	-
Sucrose	+	-
H_2S	-	+

Key: - Negative
+ Positive

Table 4: Frequency of occurrence of *E. coli* and *Salmonella* species in ready to eat vegetable salads hawked in Kaduna metropolis

Vegetable type	No. of samples examined	No. of *E. coli* (%)	No. of *Salmonella* (%)
Lettuce	40	15(37.5)	4(10.0)
Cabbage	40	9(22.5)	1(2.5)
Cucumber	40	4(10.0)	0(0)
Tomato	40	3(7.5)	0(0)
Onion	40	0(0)	0(0)
Spices	40	0(0)	0(0)
Total	240	31(12.92)	5(2.08)

DISCUSSION

The proximate composition of the sample of cabbage, cucumber, lettuce, onion, spices and tomato indicated moisture content with low composition. The result is not in line with the findings of Adeyeye (2018), who reported higher moisture content of up to 86.35 from vegetables. Similarly, a study conducted by Adeniyi *et al* (2012) also indicated higher moisture content of leafy vegetables 79.98. This might relate to the freshness of the vegetables, mode of preparation, handling and also due to the differences in the type of vegetables used. The lipid content of the cucumber and onion was low. The very low-fat contents could be advantageous for individuals suffering from obesity and other related diseases, as reported by Adeyeye (2018). The high crude fibre obtained from cucumber and lettuce contradict the findings of Adeyeye (2018) who reported low crude fiber 1.05 in vegetables. Crude fiber plays an important role in the maintenance of internal distention for a normal peristaltic movement of the intestinal tract (Balogun and Olatidoye,2012). Fiber aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a buildup and lead to several diseases. It is involved in preventing colon cancer and constipation as reported by Ajiboye *et al*. (2016).

The mean counts of lettuce, cabbage, cucumber, and tomato were above 1.00 × 10^5CFU/g. These findings were similar to that of Bukar *et al*. (2010), who recorded aerobic mesophilic count above the maximum acceptable limit set by Food and Agricultural Organization in lettuce, cabbage and tomato (1.40 × 10^6 to 1.0 × 10^7CFU/g) sourced from kwakwaci irrigation site, in fagge LGA of Kano state. Similarly, the findings were similar to that of Gbonjubola *et al*. (2012) and Chikodili *et al*, (2015) who recorded high bacterial load (6.0 × 10^4CFU/g to 2.0 × 10^6CFU/g) on vegetable salad sourced from restaurant in Zaria, Kaduna State, Nigeria. The high bacterial counts of the sample

investigated in this research could be attributed to the usage of animal dungs as fertilizers, cultivation of vegetables with sewage polluted water (domestic sewage), contact with soil and dust, poor handling and processing, use of contaminated utensils and usage of bare hands during serving of the product to the end users. However, there was no count obtained in samples of onion and spices; this could be due to their antimicrobial effects. The aerobic mesophilic bacterial counts of lettuce, cabbage, cucumber, and tomato was found to be above the maximum acceptable count of 10^3 CFU/g as reported by international commission on microbiological specification for foods.

The bacteria isolated from the vegetable samples were *Escherichia coli* and *Salmonella* species. The bacteria isolated in this study were similar to the findings of Gimba and Madueke (2015), who conducted a microbiological assessment of vegetables at Owena Ijesa of Osun State, Nigeria. These bacteria are capable of causing various types of illnesses, some of which can result in death. *Escherichia coli* was the most occurring microorganism in this study, which could be a result of fecal contamination in the wastewater. The *E. coli* is a well-established index of fecal contamination the presence in the sample may be suggestive of fecal contamination due to poor hygiene and the unhygienic condition of the water used for irrigation as reported by Slater *et al*. (2018). The presence of *Salmonella* species could be due to agricultural practices using irrigation water, untreated animal manure and also domestic and farm animals present in and near the fields and environment. This finding is in accordance with the findings of Yang *et al*. 2020 who revealed the presence of 3.4% *Salmonella* contamination in vegetables. In view of this, these ready-to-eat vegetables sold in the sampling sites can be reported as unsafe for human consumption.

CONCLUSION

There were variations in the proximate composition of the vegetables analyzed, lettuce had the highest crude fibre content, onion had the least lipid content. Lettuce had the highest mean bacterial count, while onion and spices had no count. The bacteria isolated from the vegetable salads were *Escherichia coli* and *Salmonella* speciesand as such pose substantial risk to humans as they are consumed without having undergone prior preservation or additional processing.In view of this, these ready-to-eat vegetable salads sold in the sampling site can be reported as unsafe for human consumption.

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