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INCIDENCE AND EFFECT OF PROCESSING ON *Listeria monocytogenes* LOAD OF FRESH CUT VEGETABLES

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Abstract

Fresh cut vegetable the most common food is a source of high value nutrient in human diet, processing may lead to increase microorganism in it. However, some pathogenic bacteria associated with fresh produce may cause illness, *Listeriosis.* The aim of the study was to determine the survival of *Listeria monocytogenes* and effect of processing on bacteria load of fresh cut vegetables. Processing method such as Salted Vegetable (SV), Washed Before Cutting (WBC) and Cut Before Wash (CBW) were used. Seven common vegetables (spinach, bitter leaf, scent leave, curry leaf, water leaf, fluted pumpkin, Jatropha) were screened for *listeria monocytogenes* using brilliance listeria oxoid (basingstokh Hampshire, UK). Result revealed that out of 105 composite of vegetable samples; 40 had *Listeria* sp in it leave. The incidence rate of listeria from this study was 38.1%. Water leafy had the highest incidence of listeria contamination (30%) followed by spinach (20%). Listeria count ranged from 2.39 to 4.16 and 3.30 to 3.86 log cfu/mL for water leaf and spinach. Out of the three processing methods used WBC was more effective in reducing listeria count on vegetable. It is therefore recommended that farmers should use treated waste product for vegetable planting and the imbibing of WBC method of processing for vegetables.

KEY WORD: Listeria incidence, fresh cut vegetable and method of processing

Introduction

Vegetables make up a major portion of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phyto-nutriceuticals: vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber and

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phytochemicals (Quebedeaux and Eisa 2017). Some phytochemicals of vegetables are strong antioxidants and are thought to reduce the risk of chronic disease by protecting against free-radical damage, by modifying metabolic activation and detoxification of carcinogens, or even influencing processes that alter the course of tumor cells (Van Paepeghem *et al*., 2024). Vegetables in the daily diet have been strongly associated with overall good health, improvement of gastrointestinal health and vision, reduced risk for some forms of cancer, heart disease, stroke, diabetes, anemia, gastric ulcer, rheumatoid arthritis, and other chronic diseases (Dias and Ryder 2011).

Fresh and fresh-cut produce are highly susceptible to microbial contamination due to pre-harvest and post-harvest activities Opeyemi *et al*., 2025). Pathogens on fresh fruits and vegetables are *Salmonella, Shigella, Listeria monocytogenes, E. coli 0157:H1, gastrointestinal viruses, Entamoeba histolytica,* and *Ascaris* sp.

Listeria monocytogenes, a member of the genus Listeria, has been well recognized as a foodborne pathogen and commonly found and isolated from fresh produce (Giovanni *et al*., 2026). The presence of *L. monocytogenes* in RTE products has been reported worldwide, such as in Europe, Tirloni *et al*., 2024), China (Zhang *et al*., 2021), South America and Africa (Centorotola *et al*., 2026) and Nigeria Once *Listeria monocytogenes* enter into the processing facilities, it can survive for long periods due to favorable humidity and temperature (Maggio *et al.,*2021; Yangchen *et al.,* 2025). Several outbreaks were reported, one of them lasting from 2015 to 2016 (CDC, 2011). *L. monocytogenes* is generally transmitted when food is harvested, processed, prepared, packed, transported or stored in environments contaminated with *L. monocytogenes*

In humans, the average case-fatality rate of listeriosis is between 20% and 30% even with adequate antimicrobial treatments (Romero *et al*., 2025) but can be a serious invasive disease with mortality rates ranging between 80% and 99% primarily in neonates, immunocompromised adults and pregnant women causing encephalitis, septicemia, and abortion (Dunka *et al*., 2021; Olise and Okpor, 2025). Even though, there are few published reports on the occurrence of *L. monocytogenes* in water, food samples, and environmental sources in Nigeria (Nwachukwu *et al.*2010; Okinedo *et al*., 2025), literature lacks information on the incidence of *L. monocytogenes* in vegetables that require further processing (RTE). This study therefore reports on the incidence and *L. monocytogenes* and effect of processing on *L. monocytogenes*

MATERIALS AND METHODS

This study was carried out in Bida metropolis. The study design used was descriptive study of evaluation of respondents from farmers and vegetables vendors in new market, old market, and Bangaie, Bida. The targeted population was the farmers and vegetables vendors in Bida. Included in this work were subsistence and commercial farmers as well

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as vegetable vendors excluding food and fruit vendors. The sample size was determined as stated below using Glenn's formula. Random sampling was used to determine the occurrence of *Listeria monocytogenes* on fresh cut vegetables.

A well-structured close-ended questionnaire was used to collect data from the respondents.

Sample Collection

Field composite samples (200g) of different vegetables (Spinach, ugwu leave, water leave, bitter leave curry leaves, jateropha and scent leave) were collected from three different markets and Bangaie farm located in Bida metropolis. A total of 105 samples were collected from the 4 sites. Samples were collected in sterile containers and analyzed immediately.

Sample preparation, Detection and Enumeration of *L. monocytogenes*

The detection and enumeration of the organism was carried out by the method of Mumuney *et al*. (2021). Briefly, 25 g of each vegetable was aseptically blended in a sterile Warring blender (Torrington, CT) for 5 min and pre-enriched by diluting 10 g of each lot in 90 mL of one broth Listeria (Oxoid (Basingstoke Hampshire, UK.). The mixture was vortexed for 1 min by hand inversion and incubated without agitation at 30°C for 24 h. Ten microliters of the broth culture were aseptically inoculated into Brilliance Listeria Agar (Oxoid) which was incubated at for 24 h. Suspected colonies with blue or greenish colorations were counted as *L. monocytogenes* (Opeyemi *et al*., 2025).

Methods of Processing of Vegetable

The vegetables samples were processed using four different methods and coded as below.

i. The vegetables were cut before washing - CBW.
ii. The vegetables were washed before cutting - WBC.
iii. Wash with salt after cutting vegetables -SV
iv. Vegetable without any process -Control

Data analysis

Data obtained from field work and laboratory were expressed in frequency and percentage. Analysis of variance ANOVA was carried out; the significant difference was placed at $p < 0.05$.

RESULTS AND DISCUSSION

Out of 50 questionnaires 41(82%) of the farmers were female. Those respondents that live in urban areas were higher in percentage 64.0%. Presented on Table 2 are possible sources

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of listeria contamination. Most of the respondents (51%) uses animal dump as fertilizer for the growth of vegetable. Most of the vegetable farmers use borehole water 58.9% only

Table 3 showed listeria count of log/cful/mL of spinach sample ranged from 3.30 to 3.86, 2.39 to 4.16 and 1.00 to 1.53 for water leaf and jatropha leaf respectively. Spinach and water leaf seems to have the highest listeria count log/cful/mL (3.86 and 4.16). There was significant difference (P< 0.05) among the mean count obtained from the different sample. Table 3 shows vegetable samples obtained from new market had the highest listeria count followed by Bangaie farm and old market had the least count.

Table 1: Demographic Data of the Respondent (n = 50)

Variable	Frequency	percentage%
Gender		
Male	9	18.0
female	41	82.0
Ethnicity		
Igbo	31	62.0
Hausa	13	26.0
Yoruba	2	4.0
Others	4	8.0
Resident Area		
Urban	22	44.0
Rural	28	56.0

Table 4 revealed the incidence of *Listeria* sp in vegetable, out of 105 vegetables sampled, 40 sample had listeria in it. The incidence rate of listeria from the study was 38.1%. Water leaf had the highest frequency of occurrence (30%) followed by spinach and others.

In terms of the influence of processing method on listeria count on leaf, there was significant differences (P< 0.05) among the listeria count of the processed vegetables. Samples washed with salt before cutting had the least listeria count for spinach bitter leaf, curry leaf, scent leaf, ugu leaf and jatropha followed by sample WBC.

Table 2: Source of *listeria monocytogenes* Contamination (n=50)

Variable	Frequency	Percentage (%)
Farming as occupation		
Yes	32	36
No	18	64
Owning a vegetable garden		
Yes	47	94

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Variable	Frequency	Percentage (%)
No	3	6
Type of fertilizer used		
chemical manufactured fertilizer	21	42
Animal dungs	29	58
Planting closer to a dump		
Yes	3	4
No	48	96
Type of water used		
Tap water	2	24
Well water	5	10
Borehole water	33	66
wearing of Apron		
Yes	11	22
No	38	76
Storage		
dry	12	24
In a sack	37	74
In a cloth	1	2
Washing of hand before harvest		
Yes	15	30
No	35	70
washing hands before harvest		
Yes	15	30
No	35	70
washing of hand after harvest		
Yes	49	98
No	1	2
Means of transportation		
Vehicle	30	60
Wheelbarrow	5	10
Trail	11	22
Sack	4	8

Table 3: Listeria Count of Vegetable Samples from Different Location (Log cfu/mL)

Vegetables	New market	Old market	Bangaie farm
Spinach	$3.30 + 0.09^{c}$	$3.59 + 0.02^{b}$	$3.86 + 0.03^{a}$
Bitter leaf	$2.97 + 0.08^{a}$	$2.43 + 0.02^{a}$	$1.59 + 0.79^{a}$
Scent leaf	$2.45 + 001^{b}$	$3.82 + 0.12^{b}$	$2.40 + 0.03^{c}$
Curry leaf	$3.17 + 0.12^{a}$	$1.37 + 0.68^{ab}$	$0.67 + 0.67^{ab}$

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Vegetables	New market	Old market	Bangaie farm
Water leaf	3.83 + 0.22^{ab}	4.16 + 0.02^{a}	2.39 + 0.09^{b}
Ugu leaf	4.46 + 0.79^{c}	3.45 + 0.11^{b}	3.22 + 0.04^{b}
Jatropha leaf	1.00 + 0.067^{a}	1.53 + 0.77^{a}	1.00 + 1.00^{a}

Displayed values are mean + S.E of 3 determinants, different letters of superscripts are significantly different along the row (P< 0.05).

Table 4: Incidence of *Listeria monocytogenes* in Vegetable Sample.

Vegetable Samples	Frequency of Occurrence	Percentage (%)
Spinnach	8 (15)	20
Bitter Leaf	4 (15)	10
Scent Leaf	2 (15)	5
Curry Leaf	4 (15)	10
Water Leaf	12 (15)	30
Ugu	6 (15)	15
Jatropha	4 (15)	10
TOTAL	40 (105)	38.1

Incidence of Listeria on Unprocessed Vegetables = Total No of Occurrence/Total Samples X 100 = 40/105 X 100=38.1%

Table 5 Listeria Count of Processed Vegetable Samples log cfu/mL

	CON	SV	CBW	WBC
Vegetable				
Spinach	5.08 + 0.04^{a}	1.42 + 0.06^{d}	3.50 + 0.25^{b}	2.34 + 0.15^{c}
Bitter leaf	4.02 + 0.16^{a}	0.00 + 0.00^{d}	3.35 + 0.04^{b}	1.39 + 0.05^{c}
Scent leaf	2.66 + 0.39^{a}	1.50 + 0.25^{b}	2.36 + 0.27^{b}	0.00 + 0.00^{c}
Curry leaf	3.29 + 0.44^{b}	1.27 + 0.07^{d}	3.45 + 0.09^{a}	2.25 + 0.03^{c}
Water leaf	5.19 + 0.00^{a}	5.00 + 0.09^{a}	3.16 + 0.42^{c}	4.12 + 0.00^{b}
Ugu	4.58 + 0.10^{a}	3.06 + 0.22^{b}	3.22 + 0.11^{b}	2.65 + 0.24^{b}
Jatropha	3.17 + 0.40^{a}	1.45 + 0.73^{bc}	2.79 + 0.5^{ab}	0.00 + 0.00^{c}

Values are mean I.S.E of 3 determinants. Different letters of superscripts are significantly different (P< 0.05) always the row. Key: SV = Wash with salt after cutting vegetables , CBW = cut before washing leaf, WBC = wash before cutting the leaf, Con = control (unprocessed leaf).

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Discussion

Listeria monocytogenes is a foodborne pathogen of significant public health concern due to its high environmental resilience and ability to cause severe infections in vulnerable populations (Simona *et al*., 2025). As presented in Table 1. The demographic of respondents, 44% of Vegetable vendor are predominant of urban areas. It has been reported that urban market is always wet and overcrowded with high population tours resulting in high human activities improper handling, in hygienic market practice and diverse ecological niches of the pathogen (Okorie-Kanu *et al*., 2020).

The occurrence of *listeria* sp in 40 out of 105 samples of vegetable analyzed in this study was quite alarming, knowing the health treated posted by this organism. The prevalence rate was 38.1 in this present study. The World Health Organization estimates the global prevalence of listeria to be 172,823. Other studies in Nigeria reported the prevalence of listeria in vegetable to be 27.1 in Sokoto (Mumuney *et al*., 2021) while Okinedo *et al*. (2025) documented 53.9% in Delta. The incidence of *L. monocytogenes* in leafy vegetables in the market and the sample from Bangaie farm was high and above the European Union limit of the 100cfu/g (EU 2007). The high incidence rate (38.1%) observed reported in this study may be due to the type of manual used in cultivating the vegetables. Also, the traditional practice of continual sprinkling of vegetables products (to keep them fresh in hot and dry season) with contaminated water by vendor may also be a source of contamination to the leaf. From this study 74% of Vegetable vendor use sack as a means of storage it should be ensured that the sack should be free from contaminations.

Furthermore, the incidence of *L. monocytogenes* was significantly difference ($p<0.05$) within the three locations. Bangaie had the highest incidence of *L. monocytogenes* contamination of spinach, new market had the highest incidence of *L. monocytogenes* contamination of better leaf, curry leaf and ugu, old market has the highest incidence of *L. monocytogenes* contamination of water leaf, scent leaf and jatropha.

The difference in the level of contamination of leafy from the three locations may be due to different farming practices and post-harvest handling method adopted by the farmer, the level of enlightenment by the retail seller exposure to environmental pollution and storage temperature of the leafy, since there are no known standard of preservation, selling ethics of leafy vegetables in Nigeria (Musa *et al*., 2023) spinach from Bangaie had the highest listeria count, listeria bitter leaf, ugu leaf and curry leaf from new market are as well high. Comparing the results of this study with Sant'Ana (2012) who reported 7.67 logcfu/g of *L. monocytogenes* in cabbage, it was detected much lower. However, spinach sample had 5.04log/cfu/g.

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The *L. monocytogenes* count of processed vegetable sample revealed Wash with salt after cutting vegetable had the lowest Listeria count compared to other processing methods. This may be due to the antibacterial properties of salt.

CONCLUSION AND RECOMMENDATIONS

The prevalnce of *listeria monocytogenes* of this study was 38.1%. The Listeria count of vegetable sample from the three different location was high as it exceeded the European Union limit of the 100cfu/g (EU.2007). Water leaf had the highest frequency of occurrence (30%) followed by spinach and others. In terms of the influence of processing method on listeria count on leaf, there was significant differences (P< 0.05) among the listeria count of the processed vegetables. Samples washed with salt after cutting had the least listeria count.

We therefore recommend vegetables garden should be situated away from dump site as such places are filled with microbial activities. The act of salting/washing of vegetables should be adopted as it drastically reduces *Listeria monocytogenes* count and animal dungs should be treated before using it as fertilizer as untreated animal dung is a source of *Listeria monocytogenes.*

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