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Microbial Contamination of Leafy Vegetables in Porto-Novo, Republic of Benin

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HIGHLIGHTS

- Various microbial contaminations were found in all samples of the vegetables obtained from Porto-Novo, Republic of Benin.
- *Salmonella* spp. was absent in all samples of vegetables.
- The microbial quality of leafy vegetables at Porto-Novo must be improved for reduction of health risk to consumers.

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Acronyms and abbreviations
CFU=Colony Forming Unit

ABSTRACT

Background: The vegetables provide important nutrients to human beings. Nevertheless, contaminated vegetables can cause health problems because of their microbial load. The aim of this study was to assess the microbial quality of three main leafy vegetables cultivated and consumed at Porto-Novo in Republic of Benin.

Methods: Totally, 36 samples of amaranth, nightshade, and lettuce were taken from three districts of Porto-Novo at urban gardening level. The samples were tested microbiologically according to international standards for determination of aerobic mesophilic bacteria, total coliforms, faecal coliforms, *Clostridium perfringens*, faecal streptococci, and *Salmonella* spp. The results were analyzed using SPSS software v. 16.0.

Results: In total, aerobic mesophilic bacteria counts in leafy vegetables ranged from 4.42×10^5 to 1.08×10^6 Colony Forming Unit (CFU)/g. The highest and lowest total coliform loads were found in lettuces (3.21×10^3 CFU/g) and the nightshades (1.78×10^2 CFU/g), showing significant ($p<0.05$) difference. Faecal streptococci load ranged from 1.01×10^3 to 3.18×10^3 CFU/g and was significantly ($p<0.05$) higher in amaranths than in lettuces and nightshades. *C. perfringens* ranged from 0.633×10^1 to 1.18×10^1 CFU/g. *Salmonella* spp. was absent in all vegetables.

Conclusion: High microbial contaminations were found in the three leafy vegetables in urban gardening at Porto-Novo, Benin. So, it is necessary to improve the microbial quality of leafy vegetables farmed at Porto-Novo for reduction of health risk in consumers.

Introduction

Vegetables and fruits are important components of the human healthy diet and provide fiber, minerals, and vitamins which help to prevent certain disorders and malnutrition (Nesamvuni et al., 2001; Olson et al., 2011; Steyn et al., 2001). In the same way, leafy vegetables play an important role in the diets of all populations in the world,

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particularly in Africa, Asia, and Oceania where they provide essential part of nutritional and medicinal needs (Andres and Lebailly, 2011).

A problem to these potential health benefits is that raw leafy vegetables may transmit some pathogenic microorganisms which could result in microbial outbreaks. The vegetables may be contaminated during any one of the stages of production and therefore resulted in sicknesses and outbreaks after their consumption (Beuchat, 2002; Hou et al., 2013; Lewis Ivey et al., 2012; Park et al., 2012). In this context, knowledge of the microbial quality of leafy vegetables is necessary. Thus, the purpose of this study was to evaluate the presence of microbes such as aerobic mesophilic bacteria, total coliforms, faecal coliforms, *Clostridium perfringens*, faecal streptococci, and *Salmonella* spp. in some green leafy vegetables in Porto-Novo, Republic of Benin.

Materials and methods

Sampling

The study was conducted in the city of Porto-Novo, the capital of the Republic of Benin in West Africa. Porto-Novo is located at 6°29'50'' North and 2°36'18'' East. The area of Porto-Novo is 110 km², about 1.08% of the national territory area.

Totally, 36 green leafy vegetable samples, including amaranth (*Amarantus cruentus*), nightshade (*Solanum macrocarpon*), and lettuce (*Lactuva sativa*) were randomly collected during March 2018. The mature leaves were taken from the stems, put in sterile packs, transported to the laboratory, washed, drained, and analyzed. For microbiological parameters, 12 samples of each vegetable were taken from three districts of Porto-Novo (Ouando, Akron and Akonaboè) at production level with 4 samples per district. Floristic keys were used at the National Herbarium of University of Abomey-Calavi in Benin for determining of each variety.

Preparation of samples

At laboratory level, 10 g of sample were taken and introduced into the stomacher bag. Ninety ml of Tryptone Salt (TS BK014HA; Biokar Diagnostics, France) were added and homogenized with stomacher for 2 min. The obtained solution was left for 15 min to ensure the revivification of germs stressed by homogenization.

Aerobic mesophilic bacteria

For enumeration of aerobic mesophilic bacteria, 1 ml homogenized sample was diluted aseptically and serially (10^{-1}-10^{-5}), and then, introduced into the petri dishes. Fifteen to 20 ml of Plat Count Agar (PCA Lab 149; Lab M Limited, 1 Quest Park, UK) were added (ISO 4833-1, 2013). The whole solution was homogenized by circular movements and incubated at 30 °C for 72±3 h.

Total coliforms

In order to count the total coliforms, 1 ml of each appropriate dilution (10^{-1}-10^{-2}) was inoculated into the petri dishes based on ISO 4832 (2006). Fifteen to 20 ml of Crystal Violet Neutral Red Bile Lactose agar (VRBL, BK 152 HA; Biokar Diagnostics F60000 Beauvais, France) were added. After solidification, a second layer of VRBL agar was added and incubated at 30 °C for 24 h.

Faecal coliforms

For the faecal coliforms enumeration, 1 ml of each appropriate dilution (10^{-1}-10^{-2}) was added into the petri dishes. After solidification, a second layer of VRBL agar was added and incubated at 44 °C for 24 h.

Faecal streptococci

One ml of each appropriate dilution (10^{-1}-10^{-2}) was inoculated into the petri dishes. Fifteen to 20 ml of Bile Esculin Azide agar (BEA; Scharlau 01-265 Scharlau Chemie, Barcelona, Spain) was added. The whole solution was homogenized by circular movements. After solidification, a second layer of 5 ml of the same BEA agar was added. The dishes were then incubated at 37 °C for 48 h.

Clostridium perfringens

The enumeration of *C. perfringens* was carried out in accordance with ISO 15213 (2003). One ml of each appropriate dilution (10^{-1}-10^{-2}) was added into the test tubes. Fifteen to 20 ml of Tryptone Sulfite Neomycin agar (TSN, Scharlau, 01-195; Scharlau Chemie, Barcelona, Spain) was added. The whole solution was homogenized by the vortex shaker. After solidification, a second 5 ml layer of TSN agar was added to create the anaerobiosis, and the tubes were incubated at 37 °C for 24 h.

Detection of Salmonella spp.

For detection of *Salmonella* spp., 25 g of each sample was taken, put into the stomacher bag, and then 225 ml of buffered peptone water (EPT, M614, HiMedia Laboratories Pvt, Ltd, India) was added (ISO 6579, 2017). The obtained solution was incubated for 24 h at 37 °C. Two ml of the pre-enrichment solution were collected and added separately into two test tubes containing 18 ml of

tetrathionate (Oxoid, Basingstoke, England) and 18 ml of cystine selenite (LABM, LAB 55A, International Diagnostics Group plc, England). The tubes were homogenized with a vortex mixer and incubated for 24 h at 37 °C. The cultures in tetrathionate and selenite cystine containing tubes were then seeded separately in sterile dishes containing the Hektoen agar medium (BK 067HA, Biokar Diagnostics F60000 Beauvais, France). The seeded media were incubated at 37 °C for 24 h. Characteristic colonies were identified using the API 20E system (bioMérieux, France). After 24 h of incubation at 37 °C, the strips were read according to manufacturer procedure.

Statistical analysis

Statistical analysis was done by ANOVA using SPSS software (v. 16.0). The criterion for significance was set at $p<0.05$.

Results

In total, aerobic mesophilic bacteria counts ranged from 4.42×10^5 to 1.08×10^6 Colony Forming Unit (CFU)/g in vegetables. The highest and lowest total coliform loads were found in lettuces (3.21×10^3 CFU/g) and nightshades (1.78×10^2 CFU/g), respectively, having significant difference ($p<0.05$). The comparison of faecal coliforms in the vegetables showed that amaranths and lettuces harbored significantly ($p<0.05$) higher faecal coliforms than nightshades.

Faecal streptococci load ranged from 1.01×10^3 to 3.18×10^3 CFU/g and was significantly ($p<0.05$) higher in amaranths than in lettuces and nightshades. *C. perfringens* ranged from 0.633×10^1 to 1.18×10^1 CFU/g with the contamination significantly ($p<0.05$) lower in amaranths and nightshades than in lettuces. *Salmonella* spp. was absent in all vegetables.

There was some significant ($p<0.05$) difference in microbial contamination of vegetables collected from three sampling area, including Ouando, Akron, and Akonaboè (Tables 1-3).

Discussion

In the present survey, we found considerable microbial contamination in all green leafy vegetable samples from Benin. Aerobic mesophilic bacteria are indicator showing that the samples have encountered favorable conditions for the multiplication of microbes (Aycicek et al., 2006). The results of aerobic mesophilic bacteria in our vegetable samples from Benin are compared to those reported by Kłapeć et al. (2016) in Poland (1.87×10^5 CFU/g), Maffei et al. (2013) in Brazil (10^6 to 10^7 CFU/g), and Tango et al. (2014) in Korea (1.91×10^4 to 0.23×10^6 CFU/g).

Total coliforms are used as indicators for the assessment of the hygienic conditions present in the production environments found in soil, vegetables, and in the bowels of warm-blooded animals (Nousiainen et al., 2016). Park et al. (2006) demonstrated that the detection of faecal coliforms is more appropriate rather than total coliforms for faecal pollution monitoring. Atindegla et al. (2016) reported faecal coliforms contamination ($10^8\pm1.23\times10^6$ to $10^{10}\pm1.62\times10^5$ CFU/g) in carrot, eggplant, and tomato from Southern Benin which was very higher than those found in our results. These authors mentioned that such high faecal coliforms contamination in the vegetable samples was due to using poultry manure in gardening sites.

Besides, manure is an important source of *Salmonella* spp. contamination in the vegetable samples. Similar with the present work, Kłapeć et al. (2016) found no *Salmonella* spp. in vegetables from Eastern Poland. Controversially, prevalence rate of *Salmonella* spp. in vegetables examined in Mexico was as high as 98% (Quiroz-Santiago et al., 2009). *Salmonella* spp. is facultative anaerobic, Gram-negative bacteria of the Enterobacteriaceae family with about 2500 known serotypes. *Salmonella* spp. can survive from several weeks to even two years in manure (Franz et al., 2005; Jiang et al., 2002). There are various sources of microbial contamination of leafy vegetables. At Porto-Novo in Benin, the water and the manure could be the main sources of contamination. According to Hougbenou Houngla et al. (2019), the manure used by market gardeners in Porto-Novo is from chicken (83.28%), rabbit (18.84%), pig (9.12%) and compost (38.30%). The source of water used in market gardening is swamp (79.89%), well (11.37%), borehole (6.41%), and fish breeding (2.33%). Thus, it is important to prevent contamination of vegetables with animal manure source in farm, or during processing and packaging of the fresh products.

C. perfringens count (0.633×10^1 to 1.18×10^1 CFU/g) in our vegetables was higher than those indicated in Zambia (Nguz et al., 2005) and in Eastern Poland (Kłapeć et al., 2016) where the *C. perfringens* contamination was rare in vegetable samples. *C. perfringens* is an inhabitant of environmental components, food, human, and animal bowels. So, detection of *C. perfringens* in vegetable samples of the current survey showed that there was possible risk of the disease in the local consumers in Benin.

Many pathogenic microorganisms can continue to live in the soil or on the surface of crops long enough to be transmitted to humans. The ability of microorganisms to persist for extend period depends of many factors, including structure and nature of soil, hygrometry, temperature,

light, plant nature, and competition with natural plants and animals (Leff and Fierer, 2013). Considering high contamination found in our study, some measures should be taken to prevent the risk of illnesses due to the poor microbial quality of green leafy vegetable at Porto-Novo, Benin. In this regards, it has been shown that establishment of Hazard Analysis and Critical Control Points (HACCP) on the vegetable farms could be too effective for reduction of microbial risks of fresh-cut produces (Mei Soon et al., 2012). On the other hand, it is obvious that contaminated water is a critical source of microbial contamination of vegetables. So, disinfecting of irrigation water used for primary production of vegetables in farms should be highlighted (Uyttendaele et al., 2015).

Table 1: Microbial load (colony forming unit/g) in amaranth in different districts Porto-Novo, Republic of Benin

	Ouando	**Akron**	**Akonaboè**
Aerobic mesophilic bacteria	1.78×10^{6}	$2.05\times10^{5}\pm2.52\times10^{4}$	$1.53\times10^{5}\pm2.22\times10^{4}$
Total coliforms	1.43×10^{2}	$1.35\times10^{2}\pm1.9\times10^{1}$	$4.15\times10^{3}\pm2.08\times10^{2}$
Faecal coliforms	2.75×10^{1}	6.00 ± 3.37	$2.18\times10^{3}\pm2.98\times10^{2}$
Faecal streptococci	6.98×10^{2}	$2.03\times10^{3}\pm1.71\times10^{2}$	$6.83\times10^{3}\pm3.86\times10^{2}$
Clostridium perfringens	0.075×10^{1}	0.575×10^{1}	1.25×10^{1}
Salmonella	ND	ND	ND

ND: Not Detected

Table 2: Microbial load (colony forming unit/g) in lettuces in different districts Porto-Novo, Republic of Benin

	Ouando	**Akron**	**Akonaboè**
Aerobic mesophilic bacteria	4.23×10^{5}	4.00×10^{5}	$5.03\times10^{5}\pm4.35\times10^{4}$
Total coliforms	3.20×10^{3}	2.50×10^{3}	$3.93\times10^{3}\pm6.65\times10^{2}$
Faecal coliforms	1.60×10^{2}	1.40×10^{2}	$3.10\times10^{2}\pm2.16\times10^{1}$
Faecal streptococci	2.23×10^{3}	1.08×10^{3}	$1.65\times10^{2}\pm1.29\times10^{1}$
Clostridium perfringens	1.8×10^{1}	0.95×10^{1}	0.775×10^{1}
Salmonella	ND	ND	ND

ND: Not Detected

Table 3: Microbial load (colony forming unit/g) in nightshades in different districts Porto-Novo, Republic of Benin

	Ouando	**Akron**	**Akonaboè**
Aerobic mesophilic bacteria	2.55×10^{6}	2.63×10^{5}	4.20×10^{5}
Total coliforms	8.83×10^{1}	1.55×10^{2}	2.90×10^{2}
Faecal coliforms	2.85×10^{1}	1.4×10^{1}	1.98×10^{1}
Faecal streptococci	5.08×10^{2}	2.38×10^{3}	1.53×10^{2}
Clostridium perfringens	0.925×10^{1}	0.55×10^{1}	0.625×10^{1}
Salmonella	ND	ND	ND

ND: Not Detected

Conclusion

High microbial contaminations were found in the three leafy vegetables in urban gardening at Porto-Novo, Benin. Therefore, it is necessary to improve the microbial quality of leafy vegetables farmed at Porto-Novo for reduction of health risk in the consumers. In this regard, extensive researches about microbial quality of leafy vegetables, appropriate agricultural practices, and also education/training of urban gardeners are recommended.

Author contributions

E.J.H.H. and A.G. designed the study; E.J.H.H. and C.D.T.H. conducted the experimental work and analyzed the data; E.J.H.H., A.G., and C.D.T.H. wrote the manuscript. All authors read and approved the revised manuscript.

Conflicts of interest

All the authors indicated that there is no conflict of interest in this research.

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