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A Preliminary Study on Microbial Contamination of Leafy Vegetables in Sokoto Metropolis, Nigeria

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Abstract - Five leafy vegetables commonly consumed in Sokoto metropolis were evaluated for microbiological quality using standard procedures involving standard plate count (SPC). The vegetables investigated included *Lactuaca sativa* (Lettuce), *Amaranthus hybridus* (Spinach), *Vernonia amagdalina* (Bitter leaf), *Brassica oleracea* (Cabbage) and *Moringa oleifera Lam* (Horse radish). Ten fungal species which include *Alternaria alternata, Absidia corymbifera*, *Aspergillus niger, A. flavus, Cladosporium herbarum, Fusarium oxysporium, Geotrichum candidum, Mucor racemoses, Rhizopus stolonifer* and *Trichoderma harzianum* and four species of bacteria: *Bacillus cereus, Escherichia coli, Listeria monocystogens* and *Staphyllococus aureus* were isolated from the leafy vegetables. The total colony counts of fungi ranged from 1.8 x 10^6 – 6.28 x 10^5 (cfu/g) of the vegetables while that of bacteria ranged from 1.0 x 10^6 to 2.94 x 10^6 cfu/g of the vegetables. The highest counts of microbial organisms were found on *A. hybridus* while *M. oleifera* and *B. olerarea* had the lowest fungal and bacterial counts respectively. There is a need to reduce the levels of microbial contamination on leafy vegetables through appropriate production practices, careful handling by the vendors and appropriate processing with a view to minimize the risk of human exposure to fungal and bacterial pathogens associated with the vegetables.
Keywords: Microbial Contamination; Leafy Vegetables; Fungi; Bacteria; Pathogen

Introduction

In Nigeria, leafy vegetables are commonly displayed on benches, sacks and in baskets for prospective buyers. While stored in the open markets the produce are susceptible to microbial invasion and colonization leading to contamination (Muhammad *et al., 2004*). Contamination can also occur due to pre-harvest practices such as plant fertilization with manure, sewage sludge and from irrigated water. Other aspects which contribute to microbial contamination of vegetables include injuries, high temperature and storage in contaminated bins.

The importance of microbial contamination includes reduction in the quality of the produce available for human consumption and production of toxins by the microbial contaminants which represent a health risk. The demand of fruits and vegetables has increased worldwide mainly because of rising awareness of the benefits of a healthy diet and the ease of global transportation. However, consumption of leafy vegetables is commonly viewed as a potential risk factor for infection with mycotoxigenic and enteropathogenic microbes. Many human diseases (entrities, diarrhriea, salmonellosis, aspergillosis, haemorrhagic colitis, hemolytic uremic syndrome, typhoid and dysentry) are linked to consumption of contaminated foods (FAO, 2012; Agugo and Opara, 2014.). The risk of food borne disease transmission is increased when the food materials are consumed raw or minimally processed as is common with leafy vegetables worldwide.

Estimates of production losses in developing countries are hard to evaluate. Postharvest losses of fruit and vegetables in some African countries have been estimated to reach 50% (FAO, 2008) hence minimizing post harvest losses of already produced food is more sustainable than increasing production (Kader and Rolle, 2004). Post-harvest diseases of fruits and vegetables caused by fungal and bacterial pathogens result in significant economic losses. Diseases caused by

Microbial pathogens have been a major challenge to agriculture, health as well as national economy. Bacteria and fungi are among the Microbial agents affecting vegetables, early blight also known as target spot disease incited by *Alternaria solani* is destructive and cause in reduced yield and death to the vegetable seedling (Dogondaji *et al.,* 2005).

An important preventive goal should be the rapid identification of microbial contaminants and constant reduction of conditions under which contamination of vegetables occurs. The present paper reports on the microorganisms associated with contamination of fresh leafy vegetables in Sokoto metropolis, north-western Nigeria.

Materials and Methods

Investigation was carried out in Sokoto Metropolis. The State lies between latitude 13° 3' 490N, longitude 5° 14' 890E and at an altitude of 272 m above the sea level. It is located in the extreme North Western part of Nigeria. The soil is predominantly ferruginous tropical type, texturally sandy and pH of the soil ranges between 6 and 7. Rainfall starts late from June and ends early, in September. The highest temperatures of 45°C during the hot season are experienced in the months of March and April. Harmattan, a dry cold and dusty condition is experienced between the months of November and February (Udo and Mamman, 1993).

Collection of samples and basal media

Fresh leaf samples of *Lactuaca sativa, Brassica olaracea, Amaranthus hybridus, Moringa oleifera and Varnonia amagdalina* were collected randomly from different marketing sites located in Sokoto metropolis, north-western Nigeria between the months of July and August, 2013. The samples were kept separately in clean sampling bags and transported to the mycology laboratory of Usmanu Danfodiyo University, Sokoto within 24 hours of collection for microbial analysis.

The media used for microbial isolations were Potato dextrose agar (Difco) for fungi, Nutrient agar and MacConkey agar (oxoid) for bacteria. They were prepared according to the manufacturers' instruction.

Isolation of microbial contaminants from vegetables

Ten gram (10g) of each of the leafy vegetable was suspended in 90ml of sterile distilled water in 25ml conical flask to prepare the initial dilution from which subsequent serial dilutions of the samples were made. One ml of the serially diluted samples (10^{-4} - 10^{-6}) were inoculated onto nutrient and potato dextrose agar plates and incubated at 37°C for 48hrs and 28°C for 72hrs for bacterial and fungal growth respectively. The total bacterial and fungal counts for each sample were determined using a digital illuminated colony counter (Gallen Kamp Co. Ltd) and populations expressed as colony forming units per gram of the samples.

Microbial colonies developing on the agar plates were subsequently isolated and continuously sub-cultured to obtain pure cultures of the isolates. Pure cultures of the fungal and bacterial isolates were identified based on morphological, cultural and biochemical characteristics including pigmentation, colony morphology, spore characteristics, nature of mycelium and gram stain reaction (Mackie and Mccartney 1999; Barnetth and Hunter 1998). Plates were inoculated in triplicate and the mean value was taken. Means were separated using LSD, were necessary the means were compared by one way ANOVA using SPSS (2006).

Results and Discussion

The total fungal counts obtained from the leafy vegetable samples analyzed in this study ranged from 6.8×10^6 to 1.8×10^6 cfu/g of the vegetables. The highest count being obtained from *Amaranthus hybridus* and lowest on *Moringa oleifera* with a significant difference among the vegetables (Table 1) the levels of bacterial contamination varied between 1.0×10^6 to 2.95×10^6 colony forming units (cfu) per gram of the vegetables. There were no significant differences amongst the vegetable types with regard to degree of bacterial contamination. The presence of fungi and bacteria was observed in all the leafy vegetable samples. Ten fungal species were isolated from the leafy vegetables of which *F. oxysporum, A. niger, Aspergillus flavus* and *C. herbarum* were more abundant than the other fungal species with 15%, 14% 12% and 12 % frequencies of occurrence respectively. *Trichoderma harzianum* was the least encountered fungus having 4% frequency of occurrence (Table 2). A total of four bacterial species: *Escherichia coli, Staphylococcus aureus, Listenia monocytogen and Bacillus cereus* were recovered from the vegetables (Table 3). All the four bacterial species were found to be present on Lettuce and Cabbage, *Amaranthus* contained S. *aureus* and E. *coli.* While *Moringa* and *Vernonia* contained *S. aureus* only.

Table 1. Total Viable count of microbial contaminants on leafy vegetables

Vegetables	Fungi (cfu/g x 10^6)	Bacteria (cfu/g x10^6)
Lactuaca sativa	3.44^b	1.68
Brassica olarace	2.68^c	2.00
Amaranthus hybridus	6.28^a	2.95
Moringa oleifera	1.80^d	1.00
Varnonia amagdalina	2.60^c	1.2
L.SD	0.51	---

Table 2. Occurrence of fungi associated with contamination of leafy vegetables

Fungi	Number of occurrences
Alternaria altanata	68
Absidia corimbifera	108
Aspergillus flavus	117
Aspergillus niger	137
Cladosporium herbarum	117
Fusarium oxysporum	147
Geotrichum candidum	59
Mucor recemesus	98
Rhizopus stolonifer	68
Trichoderma harzianum	39

Table 3. Distribution of fungi on leafy vegetables

	Vegetables				
Fungi	Lettuce	Cabbage	Spinach	Moringa	Vernonia
Alternaria alternata	+	+	+	-	-
Absidia corimbifera	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Cladosporium herbarum	+	+	+	+	+
Fusarium oxysporum	+	+	-	-	+
Geotrichum candidum	+	+	-	-	-
Mucor recemesus	+	+	+	+	+
Rhizopus stolonifer	+	+	+	-	-
Trichoderma harzianum	+	+	+	-	-

+ = Present, - = Absent

Table 4. Bacterial contaminants of fresh leafy vegetables

	Vegetables				
Bacteria	Lettuce	Cabbage	Spinach	Moringa	Vernonia
Escherichia coli	+	+	+	-	-
Staphylococcus aureus	+	+	+	+	+
Listenia monocytosen	+	+	-	-	-
Bacillus cereus	+	+	-	-	-

+ = Present, - = Absent

Results show that ten fungal species: *Alternaria alternate*, *Absidia corymbifera*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium herbarum*, *Fusarium oxysporium*, *Geotrichum candidum*, *Mucur racemosus*, *Rhizopus stolonifer* and *trichoderma harzianum* and four bacterial species: *Escherichia coli, Staphylococcus aureus, Listenia monocytosen* and *Bacillus cereus* were associated with the contamination of *Lactuaca sativa* (Lettuce), *Amaranthus hybridus (*Spinach*)*, *Vernonia amagdalina* (Bitter leaf), *Brassica oleracea* (Cabbage) and *Moringa oleifera Lam* (Horse radish) leaves in Sokoto. The result is in consonant with the finding of Ibeyessie (2007), who studied the vegetable production by waste water, the presence of wide range of microbial organisms in these products showed a relationship between the microbial quality of the water used for the vegetables and the extent of human and other animals defecation, agricultural activities as well as transportation, packaging, marketing, processing before the final consumers. With regard to degree of contaminations (cfu/g), the levels for contamination exceeded the tolerance limit (10^5 cfu/g) recommended by the international commission of microbiology specification for foods (Ibrahim *et al.,* 2011). High counts also foretell the likelihood of spoilage, because most vegetables ranged between 6.8 $\times 10^6$ to 1.8 $\times 10^6$ cfu/g.

Fresh fruit and vegetables can be contaminated with a wide range of microbial organisms from the environment. Contamination can occur at different stages of production, during processing and in storage (Kim, 1993). Similarly, inappropriate handling, packaging at the retail or wholesale outlets may predisposed the vegetables to increased invasion and subsequent contamination by fungal and bacterial species especially under ambient tropical condition typical of marketing site in Sokoto metropolis.

Similar fungal and bacterial species were obtained from the different vegetables across the markets, this could be attributing to the uniformity in climatic and environmental factors in the study area. The presence of *Aspergillus* spp. in the vegetables is an indication of possible health hazards. Many strains of *Aspergillus* are known to cause food intoxication and food infection (Muhammad *et al.,* 2004). However, the difference in the occurrence of the microbial contaminants is a reflection of the genetic and physio-chemical chemical of the different plant species investigated. Fresh produce may be infected by microbial organisms at harvest, the infection may be obvious or latent, and is often associated with poor pre-harvest management such as soil, faeces, irrigation water, fungicide and insecticide handling.

It is common practice in Nigeria to display fruit and vegetable in the markets and along the streets of the vendors for prospective customers to purchase. This practice may predispose the fruit and vegetable to contamination by a wide variety of microbes present in the production environment in the market or elsewhere. Contamination with the fungi and bacteria may also be due to post-harvest factors such as faeces, human handlings, harvesting equipment, transportation and packaging, wild and domestic animals, insects, dust, rinse water, previously contaminated materials which contributes to losses during storage before consumption (Chinoko and Naqvi 1989). The vegetables could become contaminated with microbes through factors such as coughing, inadequate hygienic condition as they are exposed or left open in unsuitable marketing site for buyers and frequent handling and washing by vendors. Cross contamination of food during marketing has been identified as an important factor associated with food borne illness (Kim, 1993) It is known that low temperatures reduce respiration and other enzyme reactions which can cause deterioration and growth of the decay causing micro-organisms. Fontern (1993) found that fungi grow best at temperatures optimum for tomato ripening and cause maximum decay at 24 °C in the Cameroun. In Nigeria, temperature optimum for tomato ripening ranged between 30 and 35 °C, which corresponded with maximum rot indices as observed in this study. The vegetables were affected by chilling injury at 0-5 °C because the tissue lost its firmness after storage at these temperatures. Therefore, temperature and relative humidity management are important in reducing physiological deterioration and preventing moisture loss and shrivelling of vegetables. It is obvious that the manipulations of temperature and relativity humidity are important in controlling vegetables contamination.

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Conclusions

The investigation indicates that fungi and bacteria are associated with contamination of leafy vegetables. Thus effective control measures such as appropriate handling, packaging, transportation as well as adequate knowledge of the epidemiology of these microbes are recommended. These are with a view to minimise the adverse impact they have on the affected vegetables and subsequent consumers, as such of these microbes have been linked to production of toxins which when ingested are harmful to human and animals.

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