Microbial quality of some leafy vegetables sold in Iree

Efunwole, O. O., Adebayo.O. R.,Adedokun O. A. and Alaje, D. O.
Osun State Polytechnic, Iree, Nigeria

Abstract
This study investigates the microbial quality of some common leafy vegetables sold in Iree. Three leafy vegetables were used which were Amaranthusviridis ('Tete'), Corchorusolitorus ('Ewedu') and Talinumtriangulare ('Gbure'). The samples were bought from the Iree main market and packaged in a sterilized aluminium covered plates and were transferred immediately into the laboratory where they were examined for Total viable counts, coliform counts and Total fungal counts. The average total viable counts was 2.2 x 10^5(plate count agar) and 2.2 x 10^5 (Nutrient agar) for Amaranthusviridis, 2.1 x 10^5(Plate count agar) 2.3 x 10^5 (Nutrient agar)cfu/mL for Corchorusolitorius and 1.9 x 10^5 (plate count agar) 2.1 x 10^5(Nutrient agar) for Talinumtriangulare. Seven bacteria belonging to five genera and three fungal Spp. were isolated, which include, Staphylococcus aureus,Bacillus subtilis, Bacillus cereus, Salmonella enteritidis, Enterobacter aerogenes, Escherichia coli, Micrococcus luteus, Aspergillus niger, Aspergillus flavus, Peniciliumitalicum respectively. Staphylococcus (26%) was the most predominantly isolated followed by Bacillus subtilis (13%), Escherichia coli (12%), Streptococcus and Micrococcus (11%), Enterobacteriacea (9%), fungal (10%) and Bacillus cereus was the least (8%). These results showed that agricultural practices on these vegetables, like transportation, irrigation and even fertilizer application method could pose risks to the consumers, it is therefore advised that proper cooking of these leafy vegetables by boiling in 100^{o}C boiling water, should strictly be adhere to before consumption not just mere blanching.

1. Introduction

Iree (also Ire or IreeAlalubosa) is a Yoruba town in the north-eastern part of Osun State, Nigeria, West Africa. Its geographical coordinates are 7.55 North and 4.43 East. Iree is one of the major towns in the Boripe Local Government Area of Osun State. Iree and many other towns in the same region have their names beginning with the letter 'I' and so, are usually described as being located in the 'I' District. Such towns include Ikirun, Iba, Illa-Orangun, Inisa, Iragbiji, Iresi, Itake and Imoleke. Iree is also situated in a valley amidst seven prominent hills which, in the past, have served as natural fortresses in times of war — primarily during the Yoruba civil wars of the nineteenth century and most especially, from hostile neighboring communities. The seven hills are: Eru Hill, Ilako Hill, Ipole Hill, Adanimole Hill, Aganna Hill, Apo Hill, and Maye Hill. The 7-hill topography of the town makes it a beautiful attraction to tourists and the warm hospitality of its people makes a visit to Iree a pleasantly memorable experience that brings tourists back there time and again.

Vegetables have many classifications or descriptions. Nevertheless, despite the wide variety of botanical structures, vegetables all possess the same general nutritive properties, at least in qualitative terms. Fruits and vegetables are a major source of macronutrients such as fiber and micronutrients such a minerals and vitamins C ,thiamin, riboflavin, B-6, niacin, folate, A, and E. Phytochemicals in fruits and vegetables, such as polyphenolics, carotenoids, and glucosinolates, may also have nutritional value. While many fruits and vegetables are consumed primarily in their fresh state, some produce such as tomatoes, snap beans, corn,peaches, nectarines,and pineapples are also consumed to a significant degree in their processed state. Vegetables are botanically different and their climatic and cultural requirements are different.

According to Hill (1972), vegetables can be grouped into three broad classifications based on their botanical parts that are edible and location with respect to the ground. These are the earth vegetables, herbage vegetables, and fruit vegetables. Earth vegetables are those in which the edible parts are below the ground including modified roots and stems. Herbage vegetables are those with above ground parts including stems, leaves, buds and flowers. Fruit vegetables are those in which the botanical fruits are usually cooked and rarely eaten raw except in salads. He gave examples of crops under each of these three types of vegetables. However, these types are only broad generalizations. It is noted also that he did not include seed vegetables.

HarperCollins and Lucy Peel (2004) grouped different vegetables into the following eight main types: (1) salad vegetables, (2) fruiting vegetables, (3) squash vegetables, (4) shooting vegetables, (5) leafy vegetables, (6) pod and seed vegetables, (7) bulb vegetables, and (8) root vegetables. However, it is not clear on what basis are these groupings made. Rimando (2004) simply defines vegetables as "crops usually grown for culinary purposes." He gave examples of vegetable crops under the following classifications: (1) leafy vegetables, (2) cole crop or crucifers, (3) root and bulb crops, (4) legumes or pulses, (5) solanaceous vegetables, and (5) cucurbits. It is to be noted that these are a mixture of types of vegetables based on plant parts that are edible and on botanical family.

To put more details on Hill's types of vegetables, six vegetable types are enumerated below. The classifications are based on the botanical plant parts that are edible and used in culinary preparation. These can also be grouped into two main divisions: vegetables with consummable vegetative parts (root, stem, and leafy vegetables) and those with edible reproductive parts (flower, fruit, and seed vegetables). Based on their edible botanical parts, vegetables can be grouped into the following:

1. Root Vegetables – plants which are sources of edible roots, mainly modified underground roots, e.g., beets. These modified roots are either tuberous roots or fleshy roots.

2. Stem Vegetables – plants which are sources of edible botanical stems which are usually immature and succulent. They can be divided further into those plants in which the edible plant structure consists mainly of aerial stems, e.g., bamboo shoot and those with underground modified stems such as corms, tubers, bulbs, rhizomes, and stolons.

3. Leafy Vegetables – plants which are sources of edible leaves in various stages of development. The leaves may be separate and fully expanded or, as in cabbage, form a head. It includes, in some species, succulent stems, e.g., sweet potato (camote tops).

4. Flower Vegetables – plants which are sources of edible flowers or inflorescences including, in some species, accessory parts like the stalk (pedicel or peduncle). In cauliflower, it consists of the stalk and an immature inflorescence. But in squash, it is the mature flower.

5. Fruit Vegetables – plants which are sources of edible botanical fruits, either simple or compound, e.g., tomato and corn (maize)

6. Seed Vegetables – plants which are sources of edible botanical seeds, mostly under the family Leguminosae or Fabaceae (legumes), e.g., mung bean.Nutritional policies have strongly promoted the consumption of diet containing more than 400/g of fresh vegetables and fruits as nutritional goal for health promotion (FAO/ WHO 2004),whereas the health of

the consumers can adversely be affected, if contaminated vegetables are consumed. Meanwhile contamination of vegetables can occur directly or indirectly through contact with soil, dust water and during cultivation, harvesting or storage, transporting and marketing (Eni *et al.*, 2010). Therefore, the objective of this study is to determine the microbial loads of some of the leafy vegetables that are sold in Iree, as they are the cheap source of soup for the students.

2. Materials and Methods

Collection of samples

Each of the three leafy vegetables (*Amaranthusviridis* ('Tete'), *Corchorusolitorus* ('Ewedu') and *Talinumtriangulare* ('Gbure'))was bought from Iree on the market day from three different sellers in triplicates making nine samples. Each vegetable sampled was placed separately in sterilized aluminium plates and transported to the laboratory immediately.

Microbiological Analysis

Microbial load determination

Each leafy vegetable sample was carefully dipped inside sterile water for 5 minutes by splashing inside the water to avoid the roots coming in contact with the sterile water. Each homogenate was serially diluted. At dilution of 10^5, 0.2ml of the dilution was plated in duplicate on to different media plates. Nutrient agar for total viable count, Endo agar (fluka) for coliform count and potato dextrose agar (fluka) for fungal isolation. All the media were prepared according to manufacturer's instruction. All inoculated plates were allowed to solidify, then inverted and incubated at 37^0c for 24 hour, but potato dextrose agar plates were incubated at 25-28^0c (room temperature) for 72 hour.

Purification of isolates

Distinct discrete colonies on the different media were isolated and purified on their respective agar by repeated sub-culturing.

Characterization and identification of isolates

Identification of Fungal isolates

Identification was based on their morphological, macroscopic and microscopic characteristics as seen in culture plates.(Fawole and Oso, 2010)

Identification of Bacterial isolates

Cultural characteristics and biochemical tests, IMVIC test, carbohydrate utilisation, gelatin liquefaction, nitrate reduction, motility, oxidase and urease production were carried out according to Olutiola*et al.*, (2000).

Coliform organisms

On Endo- agar coliform organisms and surrounding medium appear red while others appear colourless.

3. Results and Discussion

Average total viable count were presented in the figure below. The viable counts o ranged from 1.3 to 2.2 x 10^5Cfu/ml for the three samples. However, *T. triangulare* had the least count on Nutrient agar plates as seen in figure 1.

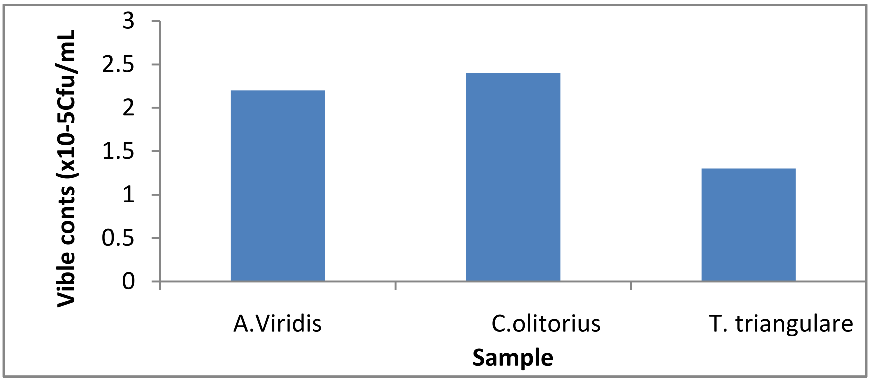

Figure 1: Viable microbial counts associated with each vegetable sample

Occurrence of each isolate

Staphylococcus aureus had the highest occurrence (26%) among all the isolates followed by *Bacillus subtilis*. However, *Bacillus cereus* had the least followed by *Enterobacter aerogenes*(9%) as presented in figure 2

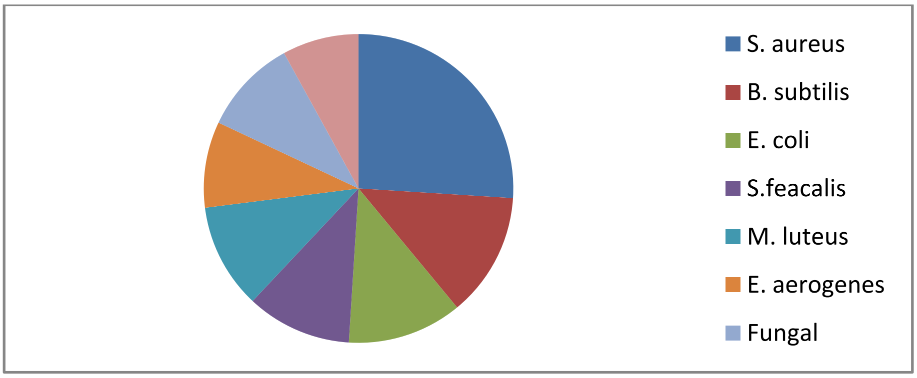

Figure 2: Percentage occurrence of each isolate on the sampled vegetables

4. Discussion

Fresh vegetables and herbs, including those of the leafy variety, have been implicated as vehicles for the transmission of microbial foodborne disease worldwide (Beuchat, 2006). *E. coli* is a more specific indicator of faecal contamination. Mukherjee *et al.*, (2004) found *E. coli* in 10.7% (9/84) of field samples of leafy vegetables, with 22.4% (12/49) lettuce, 10.2%(4/39) cabbages and 13.3% (2/15) bokchoi contaminated. For example, leafy crops fertilized with inadequately composted manure and those fertilized with animal manure were found to have a higher risk of *E. coli* contamination (Mukherjee et al., 2004; Mukherjee, Speh and Diez-Gonzalez, 2007 De Roever,1998;).

The kind of water used during irrigation determines the type of microorganism associated with the leafy vegaetables. A range of microbiological hazards can be transmitted to humans through contact with or the ingestion of contaminated water. Excreta-related bacterial species (including *Salmonellaenterica*, *E. coli* O157:H7, *Campylobacter* spp. and *Yersinia* spp.), intestinal helminths (*Ascarislumbricoides*, *Trichuristrichuria*), amoebae (*Entamoeba coli*) and protozoa (*Giardiaintestinalis*, *Cryptosporidium parvum*, *Toxoplasma gondii*, *Cyclosporacayetanensis*) are associated with recurrent outbreaks of disease in different parts of the world (WHO, 2006).Farm workers may also be sources or vehicles for contamination of produce in the growing field. Foodborne outbreaks have been attributed to poor hygiene practices of food handlers.Machinery and equipment were also considered to have the potential to transfer microbialhazards from contaminated areas to growing fields.

5. Conclusion

Having discovered that agricultural practices on these vegetables, like transportation, irrigation and even fertilizer application method could pose risks to the consumers, it is therefore advised that proper cooking of these leafy vegetables by boiling in 100^{o}C boiling water, should strictly be adhere to before consumption not just mere blanching.

References

Beuchat, L.R. 2006.Vectors and condition for pre-harvest contamination of fruitsand vegetables with pathogens capable of causing enteric diseases.*British Food Journal*, 108: 38–53. DeRoever.C (1998).Microbiological safety evaluationsand recommendations on fresh produce. Food Control,9:6, pp.321-347.

Eni, A.O., Oluwawemitan I.A. and Oranusi, U.S. (2010). Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. Afr. J. food Sci., 4(5): 291-296.

HILL A. (1972).Economic Botany. 2nd ed. New Delhi: Tata McGraw-Hill Pub. Co. Ltd. pp. 359-384.

Mukherjee, A., Speh, S. &Diez-Gonzalez, F. 2007. Association of farmmanagement practices with riskof *Escherichia coli* contamination in pre-harvest produce grown in Minnesota and Wisconsin. International Journal of Food Technology, 120, 296-302.

Mukherjee, A., Speh, D., Dyck, E. &Diez-Gonzalez, F. 2004. Pre-harvestevaluation of coliforms,*Escherichia coli*, *Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *Journal of Food Protection*, 67(5): 894–900.

PEEL L. (2004). HarperCollins Practical Gardener: Kitchen Garden. New York, NY: HarperCollins Pub. Inc. 176 p.

RIMANDO TJ. 2004. Lecture Syllabus: Crop Science 1. College of Agriculture, UPLB. P. 15.(Ben G. Bareja, June 16, 2015)

WHO. 2006. WHO guidelines for the safe use of wastewater, excreta and greywater. Wastewater use inagriculture.WHO, Geneva, Switzerland.