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Microorganisms Associated With Post Harvest Spoilage of Lettuce (*Lactuca sativa*) and Waterleaf (*Talinum fruticosum*)

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Abstract

Microorganisms associated with the spoilage of lettuce (*Lactuca sativa*) and waterleaf (*Talinum fruticosum*) in Fruit garden and Mile 3 market in Port Harcourt metropolis was investigated. This was carried out by isolation and identification of bacteria and fungi in the spoilt vegetables and conducting Koch's postulate to prove that the microorganisms isolated actually caused the observed spoilage. The aim of this research is to ascertain the microorganisms associated with spoilage of lettuce and waterleaf by the isolation of bacteria and fungi from spoilt lettuce and waterleaf and further investigating if the isolated organisms would still cause spoilage of healthy vegetables. The study will also ascertain whether the organisms isolated are of potential health hazards to humans. The spoilage of vegetables leads to financial loss to the farmer; this loss can be reduced by preventing the spoilage of these vegetables. Understanding the microorganisms responsible for the spoilage of vegetables will go a long way in assisting vegetable farmers, traders and consumers in minimizing vegetable loss due to microbial spoilage and associated health hazards. The mean values of total aerobic heterotrophic bacteria count for lettuce ranged from 1.0×10^6 cfu/g to 1.96×10^6 cfu/g while mean values of counts in waterleaf ranged from 1.32×10^5 cfu/g to 2.0×10^6 cfu/g. The bacteria isolated were *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Shigella* species and *Staphylococcus aureus*. While the fungi isolated from the vegetables were *Aspergillus niger*, *Mucor* spp., and *Penicillium* spp. *Aspergillus niger* is a predorminant fungal specie associated with both lettuce and waterleaf. The presence of these microorganisms in the vegetables is an indication that the vegetables are contaminated with enteric organisms.

Keywords: Vegetables, microbial spoilage, diseases, economic loss.

Introduction

Lettuce (*Lactuca sativa*) plant is grown as an edible plant leaf eaten raw in salads. It can be plagued with numerous nutrient deficiencies, as well as insect and mammal pests and fungal and bacterial diseases. Depending on the variety, lettuce is an excellent source (20% of the Daily Value, DV, or higher) of vitamin K (97% DV) and vitamin A (21% DV), with higher concentrations of the provitamin A compound, found in darker green lettuces, such as Romaine (Davey, 2007).

Talinum fruticosum (waterleaf) leaves are also eaten raw in salads and used in making soups. Water leaves are rich sources of vitamins, lipids and protein. *T. fruticosum* is one of the most important leaf vegetables of Nigeria. As a leaf vegetable, *T. fruticosum* is rich in vitamins, including vitamins A and C, and minerals such as iron and calcium (Fasuyi, 2006).

It is estimated that about 20% vegetables produced is lost each year due to spoilage (Jay, 2005). Many vegetables present nearly ideal conditions for the survival and growth of many types of microorganisms. Most vegetables go bad because of damage caused by microorganisms such as bacteria and fungi, enzymatic processes or bruising. Microorganisms produce deterioration through structural decay (Miedes and Lorences, 2004). Bacteria and fungi release their own enzymes as they grow, speeding up the spoiling process. Enzymes, which occur naturally in vegetables, are part of the natural aging process (Pitt *et al*., 1996; Miedes and Lorences, 2004). Enzymatic browning leads to discoloration and later, spoilage. Bruising physically alters the exterior of vegetables, which triggers enzymatic reactions.

Vegetables are frequently consumed raw without being exposed to the processes that reliably eliminate pathogens and this may lead to food borne infections (Ngugen and Carlin, 1994). Many different types of bacteria, viruses and parasites can contaminate vegetable, so there is numerous different food borne infections. For almost 100 years, vegetables contaminated in the field have been recognized as a source of human infection (Ngugen and Carlin, 1994).

The cultivation and consumption of lettuce and waterleaf in Nigeria has increased tremendously in the recent years properly due to increased awareness of their health importance. The microbiological analysis of these vegetables is therefore necessary as to ascertain the health hazards associated with improperly processed vegetables.

Materials and Methods

Collection of Vegetable Samples

Samples of Lactuca sativa (Lettuce) and Talinum fruticosum (Waterleaf) were collected from two different locations namely; Fruit garden market Kaduna Street D/ Line and Mile 3 Market Diobu all in Port Harcourt metropolis. The samples were separately collected using a sterile whirl-bag appropriately labeled, and immediately taken to the laboratory for investigation. Samples were collected monthly for a period of three months. On arrival, the vegetables were placed on a sterilized laboratory bench and allowed to deteriorate for 7-8 days. The samples of spoilt vegetables were

thereafter aseptically collected and transferred into a sterile sample bottle and labeled accordingly for further analysis. Another batched of vegetable samples were observed for both physical deterioration and microbial spoilage. Observations were recorded for each stage of spoilage with increasing number of days.

Microbiological Analysis of the Vegetable Samples
Isolation of Aerobic Heterotrophic Bacteria and Fungi

One gram (1g) of spoilt lettuce and waterleaf was weighed separately on a balance and transferred into separate 9ml of sterile normal saline and then diluted serially in one tenth stepwise movement up to 10^{-3} dilution for each sample. After the serial dilution had been completed, 0.1ml aliquot from 10^{-1} to 10^{-3} was each transferred aseptically using a 1ml pipette onto the surface of a freshly prepared sterile nutrient agar plate. The inoculum was spread evenly using a sterile glass rod spreader. The nutrient agar plates were then allowed to dry inverted and incubated at 37^{o}C for 24 hours. After incubation, number of colonies that developed was counted for each plate which yielded counts between 30-300 colonies. The average colony count of duplicate cultures was calculated and recorded from which the total numbers of colony forming unit per gram (cfu/g) of vegetable sample was calculated.

An aliquot (0.1ml) of the appropriately diluted sample was inoculated Sabouraud dextrose agar (SDA) plates for the isolation of fungi. The spread plate method was done using sterile bent glass spreader to spread the sample evenly on the agar plates. Cultures SDA plates were prepared in duplicates and incubated on the laboratory bench for 5 to 7 days.

Colonies which developed on SDA plates were examined and the colour and colonial morphologies or characteristics were recorded. Colonies were subcultured onto freshly prepared SDA to obtain pure cultures of fungi.

Characterization and Identification of Bacteria and Fungi in vegetable Samples

The characterization and identification of bacteria and fungi in both lettuce and waterleaf samples were determined according to the methods described by Obire and Alali (2015). Pure cultures of bacteria were subjected to the following characterization tests performed in duplicates. Gram staining, catalase test, coagulase test, urease test sugar fermentation test, methyl red test, indole test and acid gas test were carried out. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruikshank *et al.,* (1975) and with reference to Holt (1977).

Pure cultures of fungi were subjected to the following standard characterization tests performed by macroscopic examination of the colony morphology-diameter, colour (pigmentation), texture and surface appearance. While the microscopic examination of fungi was done by needle mount or wet mount method on a slide. The slides were observed under low and high power objective for sexual and asexual reproductive structures. Observations were recorded as the cultural characteristics, sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter (1998).

Pathogenicity Test

There are four criteria designed to establish a causative relationship between a microbe and a disease. The postulate formulated by Robert Koch and Friedrich Loeffler in 1884 was adopted to establish that the microorganisms initially isolated from the spoilt lettuce and waterleaf samples were indeed the causative agents of the microbial spoilage.

These four criteria are as follows; The microorganism or other pathogen must be present in all cases of the disease; The pathogen can be isolated from the diseased host and grown in a pure culture; The pathogen from the pure culture must cause the disease when inoculated into a healthy, susceptible laboratory plant or animal; The pathogen must be re-isolated from the new host and shown to be the same as the originally inoculated pathogen.

Healthy leaves of lettuce and waterleaf were washed with tap water and distilled water and thereafter the leaves were gently scratched with a sterilized needle and the test organisms (*Aspergillus*, *Mucor* and *Penicillium*) were inoculated into the scratched surface. The leaves were thereafter incubated at room temperature for 8days during which period, observations were recorded.

Results

The results obtained from the microbiological analysis of the lettuce (*Lactuca sativa*) and waterleaf (*Talinum fruticosum*). samples showed the presence of bacteria and fungi as spoilage organisms. The mean values of total aerobic heterotrophic bacteria count for lettuce ranged from 1.0×10^6 cfu/g to 1.96×10^6 cfu/g while mean values of counts in waterleaf ranged from 1.32×10^5 cfu/g to 2.0×10^6 cfu/g. The bacteria and fungi isolated from lettuce and waterleaf from the different markets is shown in Table 1. The bacteria isolated were *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Shigella* species and *Staphylococcus aureus*. While the fungi isolated from the vegetables were *Aspergillus niger*, *Mucor* spp., and *Penicillium* spp.

Isolates	**Lettuce**		**Waterleaf**	
	Mile 3 Market	Fruit Garden Market	Mile 3 Market	Fruit Garden Market
Bacteria				
Escherichia coli	+	-	+	-
Klebsiella pneumonia	-	-	+	+
Salmonella typhi	+	+	-	-
Shigella spp	+	+	-	-
Staphylococcus aureus	+	+	+	+
Fungi				
Aspergillius spp	+	+	+	+
Mucor spp	+	-	+	-
Penicillium spp	-	-	+	+

Table 1: Bacteria and fungi isolated from lettuce and waterleaf from the markets

The observed post harvest physical and microbial spoilage of lettuce (*Lactuca sativa*) and waterleaf (*Talinum fruticosum*) is shown in Table 2. It was observed that decay started in leaves inoculated with microorganisms before decay of leaves not inoculated.

Incubation Period	Lettuce		Waterleaf	
	Physical Spoilage	**Microbial Spoilage**	**Physical Spoilage**	**Microbial Spoilage**
Day 1	Fresh Vegetables	Fresh Vegetables	Fresh vegetables	Fresh vegetables
Day 2	No spoilage observed	No spoilage observed	No spoilage observed	No spoilage observed
Day 3	No spoilage observed	Dots of brown discoloration	No spoilage observed	No spoilage observed
Day 4	brown discoloration	brownish discoloration	No spoilage observed	No spoilage observed
Day 5	Slimy. Decay setting in	Brownish discoloration in 70% of leaves	Wilting of leaves	Brownish discoloration
Day 6	Very slimy with moderate decay	Decayed leaves	Wilting of leaves	Brown discoloration
Day 7	Decayed leaves	-	40% of leaves wilted	-
Day 8	Decayed leaves	-	70% of leaves wilted	-

Table 2: Observed post harvest physical and microbial spoilage of lettuce (*Lactuca sativa*) and waterleaf (*Talinum fruticosum*)

Discussion

The present study has revealed the presence of both bacteria and fungi in lettuce (Lactuca sativa) and water leaf (Talinum fruticosum) purchased from fruit garden market D/Line and Mile 3 market Diobu all in Port Harcourt metropolis. The high count of total aerobic heterotrophic bacteria that was recorded in both vegetables is indicative of bacterial contamination. This is attributed to poor hygienic practices of farmers and marketers and other handlers.

The bacteria isolated were *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Shigella* species and *Staphylococcus aureus*. These bacteria with the exception of *Klebsiella pneumonia* and *Staphylococcus aureus* are enteric bacteria and the presence of *E. coli* indicates faecal contamination of the vegetables. This is not surprising since toilet facilities are lacking in most communities and it is common knowledge that farmers discharge human wastes on the farms. All the bacteria isolated are potential pathogens capable of initiating various types of diseases and outbreak of serious food borne illness. All the bacteria isolated in this study have also been reported by Hung *et al*., (2004). Farms differ in water sources used and production methods. Water used on the farm for irrigation considered not fit for the purpose according to National agricultural irrigation standards (Korsten, 2016), with *Escherichia coli* levels resulting from either a highly contaminated water or improper storage of water. Rosenblum *et al*., (1990) also stated that many of the microorganisms on vegetables which have caused food poisoning are derived from human faeces.

The fungal genera isolated from the vegetables were *Aspergillus*, *Mucor* and *Penicillium*. Certain species of these genera are capable of producing mycotoxins which can cause a variety of ill effect in human from allergic responses to cancer (Pitt *et al*., 1996; Chukwuka *et al*., 2010). Fungal occurrence in the vegetables of the present study therefore also constitutes public health hazards.

This study showed that lettuce recorded more bacterial count than waterleaf. Lettuce was also found to undergo both microbial and physical spoilage faster than waterleaf (Table 2). This showed that, lettuce has a shorter shelf-life than waterleaf which if not consumed after 2days of purchase will undergo both physical and microbial spoilage.

Fresh vegetables are among the more challenging of food products to commercially produce and distribute (Watada *et al*., 1984). The incidence of microorganisms in vegetables reflects the sanitary quality of the processing steps and the microbiological condition of the raw product at the time of processing (Ngugen, 1994). Fruit garden market had a better sanitary condition than Mile 3 market and this is reflected in the microbiology of the vegetables in Mile 3 market which recorded more of both bacterial and fungal isolates than the Fruit garden market.

The causative relationship in the pathogenicity test was established, because the microorganisms which were inoculated on the healthy vegetable were able to cause spoilage. The spoilage microorganisms reported in this study must have been introduced into the vegetable from contaminated farm soils, during growth in the farm, during harvesting and post harvest handling, or during storage and distribution as reported by Barth *et al*., (2009) and Lelieveld *et al*., (2003).

This study has shown that vegetables should be properly processed under hygienic conditions and consumed immediately after purchased to avoid the proliferation of spoilage microorganisms deleterious to human health.

Conclusion

The present study showed microbial contamination as one of the factors responsible for the post harvest spoilage of fresh lettuce (*Lactuca sativa*) and water leaf (*Talinum fruticosum*) samples obtained from markets in Port Harcourt. The bacteria and fungi isolated are potential pathogens that can initiate food borne infections. The high incidence of microorganisms demands that farmers and other vegetable handlers should take appropriate control measures against contamination of vegetables. They should exhibit good agricultural practices (GAPs), good personal hygiene and sanitary environment to minimize the contamination and spoilage of vegetables. This will in turn increase yield and income generated from vegetables and reduce the health hazards associated with post harvest spoilage of vegetables.

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