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Isolation of Microbial Contaminants From Vegetables

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ABSTRACT

Vegetables are the edible parts of plant. Occurrence of microbial spoilage of vegetables is recognized as a source of potential health hazard to man and animals. The research focuses on isolation of microbes particularly bacteria and fungi from marketed vegetables. Samples were collected and Standard microbiological analyses were used to isolate bacteria and fungi. Eight bacterial isolates that were isolated are *Brevibacillus brevi, Bacillus subtilis, Branmehamlla cattarhalis, Escherichia coli, Salmonella typhi, Pseudomonas aruginosa, Serratia marcscen and Staphylococcus sp.* Four fungal isolates were also isolated; *Aspergillus flavus, Aspergillus niger, Penicillium sp, Saccharomyces sp.* Pepper (*Capsicum annuum*) has the highest bacterial count (6.53×10^9 cfu/ml) while shoko (*Celosia argentia*) has the highest fungal count (5.45×10^9cfu/ml). In this study, the high prevalence of fungal and bacterial contamination of these vegetables depicts unhygienic handling of these food materials at the point of cultivation, harvesting, transportation or selling. Therefore, there is need to safeguard the health of final consumers by proper washing and disinfection of these products which are consumed in their raw forms.

Keywords: *Bacterial isolates, Microbial load, Vegetables*.

INTRODUCTION

The word vegetable was first recorded in English in the early 15th century. It comes from old French and was originally applied to all plants. The word is still used in this sense in biological contexts. It is derived from Medieval Latin ***vegetabilis*** meaning "growing", "flourishing" (Wharton, 1970). Vegetables are the edible component of plant. This typically means the leaves, stems, bulbs, seeds and root of a plant. However, the word vegetable is not scientific and its meaning is largely based on culinary and cultural tradition (ICMSF, 1986; Bankefa, 2013; Akinyele *et. al.*, 2013).

Vegetables are important protective component of food that is highly beneficial for the maintenance of good health and prevention of diseases. They contain varying proportions of vitamins such as vitamin A, K, B6, provitamins, dietary minerals and carbohydrates. Vegetable contain various medicinal agents and are valued mainly for their high vitamin and mineral content (Marie *et al.*, 2013).

Studies have evaluated the association of fruit and vegetables consumption with the reduction of risk of specific diseases ((Hu *et al.*, 2014). However, vegetables often also contain phytotoxins and antinutrients, which interfere with the absorption of nutrients (Marie *et al.*, 2013). These include *a*-solanine, *a*-chaconine, enzyme inhibitors of cholinesterase, protease, amylase, cyanide, cyanide precursors, oxalic acid and others. These toxins are natural used to wipe off the insects, predators and fungi that might attack the plant (Ayanyemi, 2013).

The number of outbreaks of food borne illnesses associated with consumption of fresh vegetables has increased due to contaminants (Sengun and Karapinar,

2004). The incidence of microorganisms in vegetables may be expected to reflect the sanitary quality of the processing steps and the aseptic condition of the raw product at the time of processing (Amoah *et.al., 2007*; Ayanyemi, 2013). However, pathogenic microorganisms of human origin may also be present in minimally processed vegetables as the minimal technological processing may be unable to remove the original contamination resulting from air, soil, water, Insects, animal, workers, harvesting and transportation equipment. Certain fungi such as *Aspergillus spp*, *Fusarium spp*, and *Penicillium spp*. which are commonly occurring filamentous fungi may grow on vegetable and their growth may result in production of toxins known as mycotoxins, which may cause a variety of illness in human having allergic responses to immune suppression and cancer (Doyle, 2015).

Vegetables are frequently consumed raw without being exposed to aseptic processes that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce microbial load but cannot be considererd as the ultimate method to eliminate pathogens (Joseph, 2013). Eating or drinking contaminated foods can cause food-borne diseases. Many different types of bacteria, viruses and parasites can contaminate food, so there is numerous different food borne infections (Richard, 2013). Microorganism can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution (Akinyele, 2009). Therefore, early interventive measures during crop development and harvesting and good agricultural practices (GAP) will provide drastic reductions in yield loss due to spoilage at all subsequent steps in the food-to-fork continuum (Eckert and Ogawa, 1988; Barth *et al*., 2009).

Vegetables particularly the leafy ones have been implicated in nearly half the gastrointestinal infections caused by norovirus in the United States. These foods are commonly eaten raw and may become contaminated during preparation by an infected food handler. Hygiene is important when handling foods that are eaten raw. Such products need to be properly washed, handled and stored to limit contamination (Akinyele *et al.*, 2013).

Some microbial contaminants are capable of colonising and creating lesions on healthy plant tissue (Zhang, 2014). The occurrence of microbial spoilage of vegetable is recognized as a source of potential health hazard to man and animals. This is due to the production of toxins by the microoganisms. Therefore, bacteriological safe fruits and vegetables are essential to maximize the health benefits promised by adequate consumption of these produce. The research focuses on isolation of pathogenic microorganisms particularly bacteria and fungi marketed vegetables.

MATERIALS AND METHODS

Study Area

Akure is situated at 7.25^0 North latitude, 5.19^0 East longitude and 396 meters elevation above the sea level. Akure is a big town in Nigeria, having about 420,594 inhabitants. Owena which is located in the suburb of Owena town in Ifedore Local Government Area of Ondo-State, between latitude 7.15^0 N, longitude 5.05^0 E (Lasisi, 2002).

Collection of samples

During the period of the study, six vegetables samples; Jews mallow (Ewedu,

Corchorus sp), Lagos spinach (shoko – *Celosia argentia*), tomato (*Solanum lycopersicum*), Chili (shombo – *Capsicum annuum*) Cayenne pepper (Bawa – *Capsicum annuum*) and water leaf (*Tallinum triangulare*), were bought from local markets in different towns located in Ondo state and Osun state. These states are located in the Southwest part of Nigeria. The samples were not collected aseptically because the main purpose was to know the level of contamination associated with vegetables ranging from harvesting, handling and selling.

Sterilization of materials

The glass wares were thoroughly washed with detergent rinsed thoroughly with distilled water and then air dried. The glass wares were sterilized in the hot air oven at 160°c for one hour. The inoculating loop was also sterilized by flaming. The work bench was disinfected by swabbing with 95% ethanol. All work in the laboratory was done in a sterile environment.

Preparation of media

The media used in this research work were nutrient agar, potato dextrose agar, Skimmed milk agar, starch agar, tributyrin agar, peptone water and they were all prepared according to manufacturer's instructions. The media was dissolved in the adequate amount of distilled water. The media were all homogenized and autoclaved at 121°c for 15 minutes.

Isolation and identification of fungi

The samples were serially diluted using sterile distilled water and homogenized. The diluted samples (10^{-4} and 10^{-6}) were plated onto sterile potato dextrose agar and incubated at 25°C for 72 hours. Distinct colonies were picked and purified by streaking on the same agar. Mycelia of the isolated fungi were picked on a slide, two drops of lactophenol-cotton blue was added and covered at an angle of 60° with a cover slip. Fungi isolates were characterized and identified based on their colonial morphology and microscopic characteristics at magnification of ×40 objective lens. They were identified using different identification keys (Nelson *et al*., 1983).

Isolation of bacteria

The samples were serially diluted using sterile distilled water and homogenized. The diluted samples (10^{-4} and 10^{-6}) were plated on sterile nutrient agar and incubated at 37°C for 24 hours. Distinct colonies were picked and purified by streaking on the same agar. The pure cultures were preserved on agar slants for further studies (Bradshaw, 1979). Colony counting was done by means of a Gallenkamp colony counter.

Identification of bacterial isolates

Spore and gram staining for microscopic characterization of bacteria were carried out. Other Biochemical tests such as; sugar fermentation test, coagulase test, catalase test, Methyl red test, voges-proskeuer test, SIM test, citrate test were also used to identify bacteria isolates.

Gram staining

A loopful of young culture was placed on the slide. Smear was prepared by spreading the culture in distilled water; the smear was air dried and heat fixed. The heat fixed smear was first stained with crystal violet for 60 seconds. After washing the slide, it was stained with safranin for 60 seconds, rinsed with water and air dried. The cells were examined under the light microscope using x100 objectives lens for gram's reaction and cellular morphology (Cheesbrough, 2006).

Bacterial smear was prepared on slide. The smear was air dried and heat fixed. The primary stain malachite green was applied on the heat fixed slide and was allowed to steam in water bath for five minutes.

Malachite green stain was re-applied to avoid drying out. The slides were removed from the steam and rinsed with water until the slide was clear. The slide was flooded with counterstain safranin for 60 seconds and then rinsed with water. The slides were viewed under oil immersion lens with a light microscope.

After the staining procedure, the endospores appeared green having retained the primary stain (malachite green). Vegetative cell appeared pink having retained the counterstain safranin (Onyeagba, 2004).

Catalase Test

A drop of hydrogen peroxide was placed on a grease free clean slide, a sterile inoculating loop was used to pick a loop-full of the isolate and spread on the hydrogen peroxide. Formation of bubbles indicates the presence of the catalase enzyme (Bailey and Scott, 1974).

Voges-Proskauer Test

The test isolates were inoculated into glucose phosphate broth and incubated at 37°C for 72 hours. After incubation, 40% Potassium hydroxide (alpha-napthol) was added to the culture and observed for colour change (Macfaddin, 2000).

Methyl-red Test

The test isolates were inoculated into test tubes containing glucose phosphate broth and incubated at 37°C for 72 hours. After incubation, the methyl red indicator (0.02g in 50ml of 95% ethanol) was added to the culture and observed for change in colour. Red coloration indicates a positive result while yellow coloration indicates a negative result (Beck, 2000).

Sulphur, indole and mortility (SIM) Test

The test isolates were inoculated into test tubes containing SIM agar by stabbing to the bottom of the tube and streaked, then incubated at 37°C for 24hrs. After incubation, colour change was observed. Black coloration indicates H_2S is produced while turbidity of the organism from the stab mark indicates the organism is motile. Kovacs reagent was then added to the culture and observed for change in colour. Red coloration indicates indole positive (Janda, 2006).

Citrate Test

The test isolates were inoculated into test tubes containing Simmon's Citrate Agar by stabbing to the bottom of the tubes and streaked, then incubated at 37°C for 24hrs. After incubation, colour change was observed, blue coloration indicates the organism is citrate positive (koneman, 2006).

Fermentation of sugar

The sugars used for this experiment were glucose, lactose, sucrose, and malatose. One gram of each sugar was weighed into different conical flask. Phenol red (0.01g) was added as an indicator and 5ml each of the sugar solution were dispensed into different tubes with Durham's tube inserted into each test tube. The tubes were plugged with cotton wool and labelled appropriately; it was then sterilized in an autoclave at 121°C for 15 minutes after which the tubes were allowed to cool.

The bacterial isolates were inoculated aseptically into sugar solution in the tubes and incubated at 37°C for 72 hours. The change of colour from red to yellow indicates acid production which implies the utilization of sugar by the organism and appearance of bubble in the Durhams tubes indicates gas production. Un-inoculated tubes were used as control (Cheesbrough, 2006).

Coagulase Test

A loop-full of test isolates were picked from a young culture, emulsified with serum placed on a clean grease free slide and rocked for 1 minute. The presence of

agglutination indicates a positive reaction (Winn *et al*., 2006).

RESULTS

Table 1 shows the colonial morphology of bacteria based on the form, size, texture, colour, opacity, surface, elevation and margin. Table 2 shows the microscopy and biochemical reaction of bacteria isolated from vegetables based on gram reaction, spore formation, motility, methylred, voges proskauer, catalase, and coagulase. Table 3 and 4 shows the total bacterial and fungal count of the marketed vegetable samples calculated in colony forming units per ml. Table 5 shows the cultural characteristics and the microscopic examination of fungi isolates by using selective media, potato dextrose agar

TABLE 1: Colonial morphology of bacteria isolated from vegetables

I SOLATES ID	FORM	SIZE	COLOUR	OPACITY	SURFACE	TEXTURE	ELEVATION	MARGIN
TO 1	Circular	Small	Cream	Opaque	Glistering	Moist	Raised	Entire
TO2	Circular	Small	Yellow	Translucent	Wrinkled	Butyrous	Flat	Enitre
TO3	Filamentous	Medium	Cream	Tanslucent	Veined	Moist	Flat	Filiform
SB1	Circular	Small	Green	Translucent	Smooth	Moist	Flat	Entire
SB2	Rhizoid	Large	Cream	Cloudy	Wrinkled	Moist	Raised	Undulate
SB3	Circular	Large	Cream	Cloudy	Glistering	Moist	Raised	Lobate
SB4	Circular	Puntiform	Cream	Translucent	Glistering	Moist	Flat	Entire
EW 1	Circular	Medium	Cream	Translucent	Glistering	Moist	Flat	Entire
EW2	Circular	Medium	Cream	Translucent	Glistering	Moist	Flat	Entire
SH1	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
PE1	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
PE2	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
PE3	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
WL1	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire

KEYS: EW (Ewedu); SH (shoko); TO (Tomato); PE (Pepper); SB (Shombo); WL (Water leaf)

TABLE 2: Microscopy and biochemical reaction of bacteria isolated from vegetables

ISOLATES ID	GR	SPORE FORMERS	MOTILITY	MR	VP	CATALASE	COAGULASE
TO 1	+ Rod	+	+	+	-	+	-
TO2	+ Rod	+	+	-	+	+	+
TO3	+ cocci	-	+	+	-	+	+
SB1	- Rod	-	+	-	+	+	-
SB2	+ Rod	+	+	+	-	+	-
SB3	- Rod	-	+	+	-	+	-
SB4	+ Rod	+	+	+	-	+	+
EW 1	- Rod	-	+	+	-	+	-
EW2	+ Rod	+	+	+	+	+	-
SH1	+Rod	+	+	+	-	+	-
PE1	+cocci clusters	-	+	+	-	+	-
PE2	- Cocci	-	+	-	+	+	+
PE3	- Rod	-	+	-	+	+	-
WL1	+ cocci	-	+	-	+	+	+

Keys: Negative : - positive: + GR : Grams reaction MR : Methyl Red, VP : Voges Proskauer

Table 3: Total bacterial count of marketed vegetables

Samples	Bacterial count (10^{-4})	Bacterial count (10^{-6})
EW	1.60×10^5	2.64×10^9
SH	2.13×10^5	6.43×10^9
TO	2.55×10^5	3.23×10^9
PE	2.65×15^6	6.53×10^9
SB	2.76×10^5	5.75×10^9
WL	5.20×10^5	7.25×10^9

KEYS: EW (Ewedu); SH (shoko); TO (Tomato); PE (Pepper); SB (Shombo); WL (Water leaf); CFU/ML (Colony forming units per ml)

Table 4: Total fungal count of marketed vegetables

Samples	Fungal count ($10^{-4)}$	Fungal count (10^{-6})
EW	2.43×10^5	5.00×10^9
SH	2.63×10^5	5.45×10^9
TO	$1.1\ 3 \times 10^5$	3.61×10^9
PE	1.09×10^5	3.82×10^9
SB	1.26×10^5	3.93×10^9
WL	1.06×10^5	3.23×10^9

KEYWORDS: EW : (Ewedu); SH : (shoko); TO : (Tomato); PE : (Pepper); SB : (Shombo); WL : (Water leaf); CFU/ML : (Colony forming units per ml).

TABLE 5: Characteristics of the fungi isolates on potato dextrose agar

Isolates ID	Cultural characteristics	Microscopic examination of slide culture	Organism
TO 1	Whitish colonies becoming brown black with age	Non septatesporangiosphore are directly opposite the branched rhizoids. Sporangia are subglobose. Sporangiospores are ovoid in shape and columellaaresubglobose.	*Rhizopus stolonifer*
TO2	Wrinkled gray colony becoming with brownish gray with age.	Non septate mycelia branching sporangiophores; columellapyriform, ellipsoidal, pointed conical	*Mucor spp*
TO3	White fluffy growth of colonies with elevated mycelia that turned black after 36 hours	Black with sulphur yellow area on the surface single celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bud of the septate hyphae.	*Aspergillus niger*
SB1	Colonies are granular, velvety or wooly and yellow brown	Conodiophores are long and rough just beneath the globose vesicle. Philades are circumferential and are biseratial. Conidia are round, smooth or slightly rough and form long chains.	*Aspergillus flavus*
SB2	Whitish pink mycelial growth with colonies. Colonies are whitish-pick with micro-conidia, ovoid to mycelia growth a purple tinge myce-liumellipsodal in shape are extensive and cottony in culture	Macro conidia culture are borne on phialides on branched conidiospores. Septate fusiform, slightly curved and pointed at both ends	*Fusarium spp*
SB3	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
SB4	Powdery olivaceous green with sterile margin. Orange to red, wrinkled, radially furrowed	Conidia head has a symmetric penicillin being tangled in chains of conidia	*Penicillium spp*
EW 1	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
EW2	Powdery olivaceous green with sterile margin. Orange to red, wrinkled, radially furrowed	Conidia head has a symmetric penicillin being tangled in chains of conidia	*Penicillium spp*
SH1	White fluffy growth of colonies with elevated mycelia that turned black after 36 hours	Black with sulphur yellow area on the surface single celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bud of the septate hyphae.	*Aspergillus niger*
PE1	Colonies are granular, velvety or wooly and yellow brown	Conodiophores are long and rough just beneath the globose vesicle. Philades are circumferential and are biseratial. Conidia are round, smooth or slightly rough and form long chains.	*Aspergillus flavus*

PE2	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
PE3	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
WL1	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*

TABLE 6: SUGAR FERMENTATION PROFILE OF BACTERIA ISOLATED FROM MARKETED VEGETABLES

ISOLATES	GLUCOSE	SUCROSE	LACTOSE	FRUCTOSE	GAS	INDOLE	H_2S	PROBABLE ORGANISM
TO 1	-	-	-	+	-	+	-	*Brevibacillus brevi*
TO2	+	+	-	+	-	+	-	*Brevibacillus brevi*
TO3	+	+	-	+	+	-	-	*Staphylococcus sp*
SB1	+	-	-	+	-	-	-	*Pseudomonas aruginosa*
SB2	-	-	+	+	+	-	-	*Salmonella typhii*
SB3	+	-	+	+	-	-	-	*Salmonella typhii*
SB4	-	-	+	-	+	-	-	*Bacillus subtilis*
EW 1	+	-	+	-	-	-	-	*Escherichia coli*
EW2	-	-	+	+	+	+	-	*Escherichia coli*
SH1	-	+	-	+	-	+	-	*Paenibacillus validus*
PE1	-	+	-	-	+	-	+	*Branmehamlla cattarhalis*
PE2	+	+	-	+	+	-	-	*Branmehamlla cattarhalis*
PE3	+	+	+	+	+	+	-	*Serratia marcscens*
WL1	+	+	+	+	+	+	-	*Staphylococcus sp*

KEYS: Negative - ; Positive +

DISCUSSION

Fourteen bacteria isolates were isolated from freshly marketed vegetables which includes Jews mallow (Ewedu, *Corchorus* sp), Lagos spinach (shoko – *Celosia argentia*), tomato (*Solanum lycopersicum*), Chili (shombo, *Capsicum annuum*) Cayenne pepper (Bawa, *Capsicum annuum*), water leaf (*Tallinum triangulare*) based on colonial, morphological, sugar fermentation and biochemical tests. The presence of *Brevibacillus brevi, Paenibacillus validus, Bacillus subtilis* as also detected by Breidt (2009) revealed the unhygienic practices of the traders when handling vegetables or contamination from soil, air and dust.

The *E.coli* and *S. typhii* isolated in the study is in consonance with the study conducted by Baiyewu (1998). This may be linked to animal dung and manure used during the cultivation of vegetables as fertilizers. *S. typhii* has been implicated to be responsible for typhoid fever (Baiyewu, 1998). Most strains of *Staphylococcus spp* are known to be pathogenic due to the heat stable enterotoxin they produce. The presence of *Staphyloccus sp*, as supported by the study of Ayoola (2007) may lead to contamination of food and eventually affects the health of the consumers.

Branmehamella cattarhalis isolated from pepper (*Capsicum annuum*) may be due to air contamination. It is of high potential risk when consumed raw or under-cooked by the

consumers. It causes upper respiratory tract infection such as sinusitis and otitis (Barth, 2009).

Pseudosomonas aeruginosa is a prominent inhabitant of soil which is responsible for diseases of vegetables. It is associated with spoilage of vegetable. According to Amusa (2007) it is an important cause of infection and is a frequent cause of nosocomial infections such as pneumonia, urinary tract infection (UTIs), and bacteremia. It invades burns area, causes septic shock and responsible for cystic fibrosis in human. *Serratia marcescens*, an opportunistic human pathogen isolated in the study had also been implicated as one of the organisms causing nosocomial infection (Micheal, 2013).

According to Akinyele (2013), fungi isolate *Rhizopus stolonifer* and *Mucor mucedo* found in the study causes food spoilage. They grow on the surface of moist, carbohydrate-rich foods, such as breads, fruits and vegetables. The presence of *Penicillium sp* and *Aspergillus niger* agreed with study of (Akinyele, 2013), and could be due to the fact that these organisms are spore formers and are known as common environmental contaminants nevertheless, they have been implicated as food borne pathogens.

In this study, the high prevalence of fungi and bacteria was further enhanced by unhygienic mode of transportation of these consumable products. Also, local practice of using organic manure, such as human, animal and poultry dropping as fertilizer might have contributed immensely to the occurrence of the pathogen. The contamination of vegetables by pathogenic bacteria and fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distributions, marketing practices and transportation (Effiuvwevwere, 2000).

Consequent upon all these findings it is therefore very necessary and important for regulatory authorities in conjunction with government are to formulate a aseptic techniques of producing, handling, processing, storing and retailing vegetables especially in developing countries such as Nigeria. Vegetables should be thoroughly washed with clean portable water before consuming raw and before cooking in order to reduce food borne infections. It is therefore necessary and important that both the farmer who harvests the vegetables for transportation, the marketers and consumers take necessary and appropriate precautions in preventing contamination and eating of contaminated vegetables. We therefore recommend that government at different levels should intensify the provision of portable water in our farm settlements to reduce the use of un-treated waters for farming. Animal dung used as manure should be well treated with disinfectants or autoclaved to reduce their level of contamination of the vegetable by pathogenic organisms. Likewise, standard stalls should be erected in our market places where the sellers can conveniently display their goods without been too close to the road to reduce air borne contamination of the consumables in the markets.

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REFERENCES

Adeleke, O. E. and Odela, H. A. (1997). Plasmid profiles of multiple drug resistance local strain of *Staphyloccus aureus*. *American Journal of Medical Science*; 20 pp 111- 121.

Akinyele, B. J., Oladejo, B. O., Bankefa, E. O. And Ayanyemi, S. A. (2013). Microbiological analysis and antimicrobial sensitivity pattern of

microorganism isolated from vegetables sold in Akure, Nigeria. *International Journal of Current Microbiology and Applied Sciences*, 2(10): 306-313.

Akinmusire, O. O. (2011). Fungal Species Associated with the Spoilage of Some Edible Fruits in Maiduguri, Northern Eastern Nigeria. Advances in Environmental Biology., 5(1): 157-161.

Akintobi, A. O., Okonko, I. O., Akano, O. R., Agunbiade, S. O. and Onianwa, O (2011). Isolation and identification of fungi associated with the spoilage of some selected fruits in Ibadan, South Western Nigeria. Academia Arena, 3(11): 1-10.

Aletor, O., Oshodi, R. and Ipinmoroti, K. (2002) Chemical composition of common leafy vegetables and functional properties therir leaf protein concentrates. *Journal of food chemistry*. 78: 63-67.

Amoah, P., Drechsel, P., Abaidoo, R. C., and Klutse, A. (2007). Effectiveness of common and improved sanitary washing methods in selected cities of West Africa for the reduction of coliform bacteria and helminth eggs on vegetables. *Tropical Medicine and International Health*, 12:40-50.

Baiyewu, R. A., Amusa, N. A., Ayoola, O. A. And Babalola, O. O. (2007). Survey of the post harvest diseases and aflatoxin contamination of marketed Pawpaw fruit (Carica papaya L) in South Western Nigeria. *African Journal of Agricultural Research*, 2(4): 178-181.

Barry-Ryan, C. and O'Beirne. D. (2000). Effects of peeling methods on the quality of ready-to use carrot slices. *Journal of Food Science Technology*, 35,243-254.

Barth, M., Hankinson, T. R., Zhuang, H. and Breidt, F. (2009) Microbiological Spoilage of Fruits and Vegetables.

Beck, R. W. (2000) A Chronology of Microbiology in Historical context ASM press, Washington, DC

Winn, W. S., Allen, W. Janda, E., koneman,, G., Procop, P Schreckenberger and Woods, G. (2006). koneman's color atlas and textbook of diagnostic microbiology (6^{th} edition) pp 68-70.

Bankefa E. O. and Oyetayo V. O. (2013). Assessment of the preservative efficacy of ethanolic extract of Ficus carica on Capsicum frutescens Linn. *Journal of Biological and Food Science Research.* Vol 2(2).

Beuchat, L. R. (1998) Surface Decontamination of Fruits and Vegetables Eaten Raw: A Review. Food Safety Unit, World Health Organization. Geneva, 31-42.

Bradshaw L. J. (1979), Pour plate method in laboratory Microbiology; 3^{rd} edition W.B Squder Company of London. pp 532-540

Bulaong S. S. P., Dharmaputra O. S. (2002). Fungal Population, Aflatoxin And Free Acid Content of Peanuts Packed In Different Bag Types, Biotropia NO. 19: pp 1- 25

Burnett and Beuchat, (2001).Emerging infectious Diseases. Produce Handling and Processing Practices.; 5:6

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2 Press Syndicate of the university of Cambridge, United Kingdom pp. 63-70

Chuku, E. C., Ogbonna, D. N., Onuegbu, B. A. And Adeleke, (2008).

Comparative Studies on the Fungi and Bio-Chemical Characteristics of Snake Guard (*Trichosanthes curcumerina*) and Tomato (*Lycopersicon esculentus*) in Rivers state, Nigeria. Journal of Applied Sciences, 8(1): 168-172.

Eckert, J. W and Ogawa J. M. (1998). The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. Annual Review Phytopathology; 26, 433-469.

Effiuvwevwere, B. J. O. (2000). Microbial Spoilage Agents of Tropical and Assorted fruits and Vegetables (An Illustrated References Book).Paragraphics publishing company, Port Harcourt, (1st edition). pp 1-39.

Hu, D., Huang, J., Wang, Y., Zhang, D. and Qu, Y. (2014). Fruits and Vegetables Consumption and Risk of Stroke A Meta-Analysis of Prospective Cohort Studies. *American Heart Association.* 45(6):1613-1619.

ICMSF [International Commission on Microbiological Specifications for Foods]. (1986). Microorganisms in Foods, 2. Sampling for microbiological analysis. Principles and specific applications. 2nd Edition., *Blackwell Science Oxford. UK.*

Kader A. A. and Ben-Yehoshua, S. (2000). Effects of super atmospheric oxygen levels on post harvest physiology and quality of fresh fruits and vegetables. Post harvest Biology and Technology, 20, 1-13

Kayser, F. H., Bienz, K. A., Eckert, J. and Zinkernagel, R. M. (2005). Medical Microbiology.. Thieme Stuttgart, New York, (10th Edition), 571-578.

Lasisi, M. (2002). Owena/Ondo Water Supply Scheme. Ondo State Water Corporation (OSWC). Pp. 2-3.

Marie, V. C., Joan, V., Zarini, G. G. and Huffman, F. G. (2013). Impact of Vegetable Preparation Method and Taste-Test on Vegetable Preference for First Grade Children in the United States. *Department of Dietetics and Nutrition.* Paper 7. Pp: 315-326.

Onyeagba , A. (2004) Laboratory guide for microbiology. Crystal Publishers, Okigwe, Imo State pp. 95-97

Poorna, V. (2001). Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. International Journal of Hygiene and environmental health, 203(3):205-213.

Sengun, I. Y., and Karapinar, M. (2004). Effectiveness of lemon juice, vinegar and their mixture in the elimination of *Salmonella typhimurium* on carrots (*Daucus carota* L.). *International Journal of Food Microbiology*. 96: 301-305.

Solomon, C., Michael, U., Bitrus, J., Micheal, A., Aloysius, U., Godwin, O., Joan, P., Richard, A. and Joseph, A. (2013). Parasitological Evaluation of Domestic Water Sources in a Rural Community in Nigeria. *British Microbiology Research Journal*, 3 (3): 393-399.

Sperber, W. H. and Doyle, M. P. (2015). Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food safety. *Springer Science+Business Media, LLC*; pp 35-183.

Wharton, C. R. (1970). Subsistence Agriculture and Economic Development. *Transaction Publishers*. Pp: 12-20