BACTERIOLOGICAL QUALITY OF SELECTED FRESH VEGETEBLES SOLD AT TUDUN WADA MARKET GUSAU ZAMFARA STATE NIGERIA

BY

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(141030.023)

NOVEMBER, 2018

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1410301023

A PROJECT

SUBMITTED TO THE

DEPARTMENT OF BIOLOGICAL SCIENCES

FEDERAL UNIVERSITY GUSAU

In Partial Fulfilment of the requirements

For the Award of the Degree of

BACHELOR OF SCIENCE

MICROBIOLOGY

NOVEMBER, 2018

DECLARATION

I hereby declare that this project is written by me and it has not been presented before in any application for a Bachelor Degree except for quotations and summaries which have been duly acknowledged.

Odesomi Philemon Damilare

07/12/18

Date

November, 2018

CERTIFICATION

This project entitled "Bacteriological Quality of Selected Fresh Vegetables Sold at Tudun Wada Market Gusau, Zamfara State, Nigeria" meets the regulation governing the award of Bachelor of Science of the Federal University Gusau, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project has been dedicated to God Almighty, the author and finisher of my faith and my grandmother, late Prophetess Abigail Teniade Fagbemi (May her soul rest in peace).

ACKNOWLEDGEMENTS

I give all the glory, honour, praise and power to Jesus Christ for being my pillar, my source of strength whenever I am weak and for His love, protection, provision and guidance throughout my stay in the Federal University Gusau.

I sincerely want to acknowledge the contributions of my supervisor, Mal. Naibi Mohammed, for his support, patience and understanding. His constructive criticisms and unceasing encouragements have contributed immensely to the success of this project. I also want to appreciate the Head of Department (HOD) Biological Sciences, Dr. (Mrs.) Sa'adatu A. Shinkafi, the Departmental Project Coordinator, Mal. Surajo Moyi, and other able Departmental lecturers, Mr. Bruno I. Aguh, Mal. Bashir Y. Rini, Mal. Hassan Umar and the entire staff for their contributions towards the success of this project and the affairs of the department entirely.

I appreciate my role model, my queen, my pillar, the reason I made it this far, my mother, Mrs. Olayide O. Odesomi for her love, advice, support and provision throughout this journey. I appreciate her for the days she starved so that I could have food on my table, days she wore rags so that I could look beautiful, all the sleepless nights and endless prayers so that I could be someone worth reckoning with in life, all the insults and pains she went through just for me and my sisters to be successful in life, for this and so much more I am grateful. May God continue to preserve her in good health and grant her the grace to live long to reap the fruits of her labour. To my wonderful, irreplaceable sisters, Oyinkansola and Boluwatife, thanks for the love, support and prayers. They both are the best sisters.

I appreciate the Microbiology laboratory scientists and technicians for guidance, support and contributions towards the success of this project work. I am grateful to my friends: Adebayo, Jessy, Mercy, Mamie, Dunamis, Lynda, Francis, Jerry, Anuga, Rayyahi, Hauwa'u, Yakubu, Ayotomiwa and others for their advice, support, prayers and in all other aspects they have contributed greatly to the achievement of this feat.

ABSTRACT

Bacteriological quality of selected fresh vegetables sold at Tudun-wada market in Gusau metropolis, Zamfara State, Nigeria was investigated. Three different vegetable food samples (lettuce, cabbage, cucumber) were randomly purchased. The samples were prepared and analyzed using standard procedures. Mean viable counts of the bacteria and coliforms were determined on lettuce ($1.16x10^8$ cfu/g and $9.89x10^8$ cfu/g); cabbage ($1.38x10^8$ cfu/g and $1.15x10^8$ cfu/g); cucumber ($1.07x10^8$ cfu/g and $9.5x10^7$ cfu/g), respectively. *Bacillus* specie, *Escherichia coli*, *Staphylococcus* specie, *Enterobacter* specie and *Klebsiella* specie, were isolated from the food samples analyzed. The presence of coliform bacteria in the lettuce, cabbage, and cucumber could be as a result of poor, improper and unhygienic handling/preparation practices and feacal contamination from the food samples.

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CHAPTER ONE

INTRODUCTION

The term "Vegetables" designates an edible leaf plant or plant part commonly used for food purposes. A vegetable is a tender plant part which is not sweet and is usually flavored with salt, pepper or other condiments before eating (Okigbo, 1990). It is also an edible plant in which reserved food is stored in the roots, stem, leaves and fruits. These plants are cooked before eating or eaten raw as salad plants. A clear sharp distinction between vegetables and fruits is difficult, if not impossible, however, vegetables are rich in most of the vitamins, iron, calcium, some copper, proteins, carbohydrates, fats and minerals. The nutritional content of vegetables varies considerably; some contain useful amounts of protein though generally they contain little fat and varying proportions of vitamins such as vitamin A, vitamin K, and vitamin B6; provitamins; dietary minerals; carbohydrates and some iron contents (Thomas, 2008).

Demand for vegetable food has led to an increase in the amount and selection of different products available for the consumers (Almualla, 2010). As well as being considered low-calorie food, they are rich in fiber and provide a great variety of vitamins, minerals, and other phytochemicals (Saroj, 2006). Their consumption is encouraged in many countries by government health agencies to protect people against a range of illnesses such as cancer and cardiovascular diseases (Mercanoglu, 2011). Therefore, continued increase in the consumption of fresh meals has occurred as a result of efforts to promote better nutrition in the population (Mercanoglu, 2011).

Vegetables are known to be rich in vitamins, iron, calcium, proteins, fat and minerals, dietary fiber and other nutrients including flavinoids, carotenoids and phenoitic components that may lower the risk of cancer, heart disease and other illness (Nguyen and Carlin, 2004). During harvesting and transportation, raw vegetables may be bruised resulting in the release of plant nutrients and thereby, providing substrate for microorganisms present on the surface of the leafy vegetables to grow. In addition, the processing of fresh vegetables salad may alter or increase the number and type of pathogens present on the surface of the product.

According to the International Commission on Microbiological Specifications for Food (ICMSF, 1974) the incidence of microorganisms in vegetables may be expected to reflect the microbiological condition of the raw products at the time of processing. Raw vegetables are consumed without enough heating process, and therefore the possibility of food poisoning and food-borne infection always exist.

In case of nutrient composition, Lund (1981) gave an approximate percentage of the main food content of vegetables as having 8.6% carbohydrates, 2.0% protein, 0.3% fats, 0.9% ash and the remaining 88.3% constitute of water contents. Due to these percentage composition, vegetables are capable of supporting the growth of microorganisms such as mould, yeast and bacteria and can consequently be spoiled by any or all of these organisms. While growing, vegetables may be exposed to many source of feacal contamination. For example water from rivers, rhines and meuse on entry to the Netherland is always contaminated with large number of *Salmonella* (Kampelmacter, 1973). If water thus polluted is used for irrigation as artificial rain, which is often practiced during long period of drought as in the summer, contamination of vegetables (especially those eaten raw) treated with surface water have been reported (Geldreish, 1971). Although after contamination as described above drying may reduce the numbers of bacteria as do also rainfall and recontamination during or after harvesting. In this respect, the wetting of vegetables to help keep them fresh which generally take place shortly before sale, is of specially interest (Geldreish, 1971).

As these vegetables undergo minimal processing or do not need further preparation before consumption, they could potentially contain pathogens that form part of their microflora, posing a public health problem. Fresh vegetables can become contaminated by pathogens as *Salmonella* at any point during the food production process. During pre-harvest, contact with contaminated irrigation water, soil, manure, or fecal matter of wild animals may occur. These pathogens can both bind to plant leaves and/or be internalized via the leaves or the endophytic root system (Kutter *et al.*, 2006). During harvest, asymptomatic human carriers might contaminate the products, and at the postharvest level, products become contaminated by contact with polluted water, other asymptomatic human carriers, or the production process environment. Over the last 30 years there has been at least a 24% of increase in the average amount of fresh vegetable consumed per person in the United States of America (Pollack, 2011). Moreover, the number of gastroenteritis outbreaks caused by foodborne pathogens after consumption of raw vegetables salads and sprouts has increased worldwide (Gould *et al.*, 2013). Even though *Salmonella* is the most common cause of disease outbreaks associated with lettuce and sprouts (Lienemann *et al.*, 2011), there are other pathogens such as the Shiga toxin producing *Escherichia coli* (STEC) O157 that have been described as relevant microbial hazards (Friesema *et al.,* 2008). For example, a large outbreak of hemolytic-uremic syndrome caused by STEC O104:H4 linked to sprouts occurred in Germany (Buchholz *et al.*, 2011).

1.1 Statement of Research Problem

There is low tempo of research and reporting on the bacteriological quality of fresh vegetables sold in Gusau, Zamfara State; resulting in the paucity of information to assess the bacteriological quality, health hazards or implications and possible pathogenic potential associated with the consumption of these food produce in this part of the north, while some studies have been reported in other northern parts of Nigeria.

1.2 Justification

This research work will provide baseline information on the bacterial load and the prevalence of possible pathogenic bacteria associated with some selected fresh vegetables sold in Gusau, Nigeria, in order to provide insight into any potential health hazards or implications and pathogenic potential associated with the consumption of these commodities.

1.3 Aim of the Study

The aim of this study is to evaluate the bacteriological quality of fresh vegetables collected in Gusau Metropolis in other to determine whether these fresh foods may represent a potential risk for infection with pathogens for the consumers.

1.4 Objectives of the Study

1. To isolate and identify bacteria associated with fresh vegetables sold in Gusau metropolis.
2. To quantify the bacterial load on fresh vegetables sold in Gusau metropolis.

1.5 Hypotheses

1. There are no bacteria associated with fresh vegetables sold in Gusau metropolis.
2. There is no bacterial load on fresh vegetables sold in Gusau metropolis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Vegetables: An Overview

Vegetables are conveniently classified as herbage and fruit vegetables (Grolier, 2008). The earth vegetables assumed the form of true roots and modified stems such as root stalks, tubers, corms and bulbs. They are well protected underground and have high energy contents e.g. beets, carrots, sweet potatoes, cassava and turnips. They also include the bulbs such as onions, garlic, chive and shallot (Okigbo, 1990).

The herbage vegetables are similar to the vegetable found in food value and chemical composition but contain less carbohydrate and more water proteins, mineral salt and vitamins. They have their nutrient material in parts above the ground. The roughage or bulk mineral value is higher than that of the earth vegetables and they include water cress, asparagus, cabbage, lettuce and spinach.

The fruit vegetables are botanically fruits but are included as vegetables as they are either eaten raw in salads or cooked. They include bread fruit, okra, eggplant, cucumber, tomatoes, pumpkin and pear (Okigbo, 1990).

2.2 Importance of Vegetables

Vegetables serve as a major part of our food supply. Vegetables are known to be rich in vitamins, iron, calcium, proteins, fats and minerals, dietary fibers and other nutrients including flavonoids, carotenoids and phenolic compounds that may lower the risk of cancer, heart disease and other illnesses (Osamwonyi *et al.*, 2013). Vegetable play a vital role in human diet, supplying some of the nutrients which other food materials are deficient, for instance, onion give 188 milligrams of phosphorus and 2.1 milligrams of iron, carrot gives 4.8 grams of protein and 1.2 grams of fats (Gital, 2007).

Vegetables are also important in neutralizing the acid substances produce in the course of digestion of meat, cheese and other foods they are value as roughage which promotes digestion and helps to prevent constipation (Oyenuga and Fetuga, 2005). Vegetables are also sources of mineral elements needed by the body such as calcium and iron, they are also valuable sources of vitamins, and some like legumes are sources of protein while others such as sweet potatoes are important sources of energy being rich in carbohydrate.

Some vegetable species do not only fulfill their function of supplying nutrients in their own right as vegetables, but are also used for flavoring food to improve appetite e.g. onions and peppers. It is also interesting to note that some of the vegetables species like legumes are used as vegetables when young but when mature and dry they are regarded as major stables.

Vegetable growing also provide a means of earning a living, and as valuable source of raw materials for the vegetable industry thus providing job opportunities.

2.2.1 Nutrition

The nutritional content of vegetables varies considerably, though generally they contain little protein or fat, and varying proportions of vitamins such as vitamin A, vitamin K and vitamin B6, provitamins, dietary minerals and carbohydrates. Vegetables contain a great variety of other phytochemicals, some of which have been claimed to have antioxidant, antibacterial, antifungal and antiviral properties (Steinmetz and Potter, 1996). Some vegetables also contain fibre, important for gastrointestinal function. Vegetables contain important nutrients necessary for healthy hair and skin as well.

2.2.2 Stimulation of the immune system

The relation of single nutrients to immune function (Chandra and Sanchielli, 2004), as well as the effect of nutrient-nutrient interactions (Kubena and McMurray., 1996) in humans and in animal models has been reviewed comprehensively. Several of the vitamins associated with diets high in fruit and vegetables have been shown to improve immune status, particularly in other individuals. The human immune system has a central role in protecting against various external disease promoting factors and perhaps against malignant cells. It is a complex and highly interactive work of cells and their products. The immune system regulates itself by means of helper and suppresser cells and soluble product. Nutrient and other constituents of fruit and vegetable have the potential to affect aspect of immune system (Kubena and McMurray, 1996).

2.2.3 Antioxidant activity

Several of the antioxidant enzymes are metallo enzymes, which contain trace minerals for which vegetables and fruits are significant sources. Mitochondria superoxide dismutase is a manganese containing enzyme, glutathione peroxides are selenium dependent enzymes. Vegetables and fruits are rich sources of manganese but not typically significant source of selenium. However selenium is found in plant tissues in proportional to the mineral concentration of the soil in which the plant grows (Stein Metz and Potter, 1996).

2.2.4 Blood pressure reduction

Blood pressure control is important for the prevention of heart disease, kidney disease, and stroke, and can be influenced by numerous factors. Hypertension can be caused by atherosclerosis, imbalances in the rennin angiotensin system, and hyperinsulinemia, which increase sodium retention in the body and speeds atherosclerosis. Consequently, a general nutritional plan to minimize hypertension risk has included attainting and maintaining a healthy body weight; consuming alcoholic beverages and sodium in moderation (Dwyer, 2005). Replacing animal products with vegetable products in vegetarian diets trials reduces blood pressure in normotensive and hypertensive individuals (Margettes *et al.*, 2006). Lower intakes of fat and higher in takes of dietary fibre and materials, such as potassium and magnesium, are aspects of a high vegetable, high fruit believed to reduce blood pressure (Appel *et al.*, 2001).

2.3 Contamination of Vegetables

As soon as vegetables are gathered into boxes, lugs, baskets or trucks during harvesting, they are subjected to contamination with spoilage organisms from each other and from the containers unless these have been adequately sanitized. During transportation to the market or the processing plant mechanical damage may increase susceptibility to decay the growth of microorganisms may take place, precooling of the product and refrigeration during transportation will slow such growth.

Washing of vegetables may involve a preminary soaking or may be achieved by agitation in water or preferably a spray treatment. Soaking and washing by agitation tend to distribute spoilage organisms from damage foods to whole foods. Recirculated or reused water may moisten surfaces enough to permit growth of organisms during a holding period. Washing with detergent or germicidal solution will reduce numbers of microorganisms on the food.

Unsafe water used for rinsing the vegetables and sprinkling to keep them fresh is also a source of contamination. Other possible sources of microorganisms include soil, faeces (human and animal origin), water (irrigation, cleaning), ice, animals (including insects and birds), handling of the product, harvesting and processing equipment and transport.

In the processing plant the vegetables are subjected to further contamination and chances for growth of microorganisms and numbers and kinds of organisms may be reduced by some procedures. Adequate washing of food, as do peeling by stem, hot water or lye and blanching (Heating to activate enzymes etc.). Sweating of products during handling increases numbers. Processes such as trimming, mechanical abrasion or peeling, cutting, pitting or coring and various methods of disintegration may add contaminants from the equipment involved. In fact every piece of equipment coming in contact with food can be a significant source of microorganisms unless it has been cleaned and sanitized adequately (Frazer, 1998). Neglected parts of any food handling system can build up numbers of microorganisms to contaminant the food. Hot water blanching although reduces total number of organisms on the food may cause the buildup of spores of thermophilic bacteria, causing the spoilage of canned foods e.g. flat sour spores in peas.

Buildup of populations of microorganisms on equipment as the result of microbial growth in the exudates and residues from food and the growth of the contaminants. Not only there is the possibility of the addition of large numbers of organisms from this sources, there is also the likelihood that these will be organisms in their logarithmic phase of growth and therefore able to continue rapid growth. The effect is especially on vegetables following blanching. The heat treatment reduces the Bacterial contents considerably damages many of the surviving cells, and consequently lengthens their lag period. On the other hand, the actively growing contaminants from the equipment can attain large numbers if enough time is allowed before freezing, drying, or caning, such growth is usually the cause of very high bacterial counts.

Raw vegetables harbor a number of pathogenic microorganisms, which may be dispersed over the plants or appear as microcolonies embedded in the plant tissues (Rahman, 2012). During harvesting and transportation, raw vegetables may be bruised resulting in the release of plant nutrients, and thereby, providing substrates for microorganisms present on the surface of the vegetables to grow (Rahman, 2012).

These contaminations of vegetables can be reduced depending primarily on the use of good agricultural practices in growing the vegetables, good hygienic practices during harvesting, picking, transporting and processing particularly washing should be paramount. The importance of washing vegetable properly especially those that are eaten raw is to effectively remove from the vegetable any pathogenic microorganism which may cause infections upon ingestion. Chlorine-containing solutions or other antibacterial compound for the treatment of raw eaten vegetables can be employed to reduce the number of contaminating microorganisms in such vegetables (Lund, 1983).

2.3.1 Sources of microbial contamination of vegetables

Vegetables can be contaminated with pathogenic microorganisms as human enteric bacteria during pre-harvest (Farber, 2003), during growing in the field through contact with soil, dust, irrigation water, and manure of human and animal feaces, or during harvesting, post-harvest, handling, processing, distribution and marketing or in the home kitchen. Therefore, vegetables may act as a reservoirs for many microorganisms from which they colonize inside these vegetables and infect susceptible host.

2.3.1.1 Pre-harvest contamination

Organic fertilizer

The use of fertilizer such as animal manures, abattoir waste and sewage sludge may introduce pathogen directly to the field, and run-off can contaminate irrigation water. Animal wastes in the form of manure are largely recycled to contaminate agricultural land as the most economical and environmentally sustainable means of treatment and reuse. Many outbreaks of infection have been associated with water or food, including processed vegetables, directly or indirectly contaminated with animal manure (Farber, 2003).

Sludge from rural sewage treatment plants is spread onto agricultural land without any sanitation procedure, and this entails some risk of introducing pathogenic agents into the soil, which evaluate the health hazards to humans and animals. Controlling the levels of pathogens in animal wastes used on agricultural fields should help to reduce pathogen contamination of soil, surrounding water, and produce grown in these areas.

Irrigation water

In many countries, wastewater is used for irrigation of vegetables and also used as fertilizers. Irrigation with poor-quality water can cause a serious cases of contamination on vegetables. Groundwater, surface water, and human wastewater are commonly used for irrigation. Groundwater is generally of good microbial quality, unless it is contaminated with surface runoff; human wastewater is usually of very poor microbial quality and requires extensive treatment before it can be used safely to irrigate crops; surface water is of variable microbial quality. In addition, wastewater also contains salts, toxic metallic compounds and pathogenic organisms which may be harmful to the soil, crops, grazing animals and human health.

Microbiologically, irrigated vegetables are found to be highly contaminated with bacteria that are harmful to plants, animals and human. Contaminated irrigation water can transport pathogenic bacteria, which persists in the soil for a long period of time and contaminates the vegetables growing in the field irrigated by this contaminated water. The risk of disease transmission from pathogenic microorganisms present in irrigation water is influenced by the level of contamination; the persistence of pathogens in water, in soil, and on crops; and the route of exposure.

Soil

Pathogens are naturally present in soil, such as *Listeria* spp. (Nicholson *et al.*, 2005) as *L. monocytogenes, Clostridium* species, including *C. botulinum, C. perfringens* and *Bacillus cereus* or may become incorporated in the soil matrix from organic wastes added as fertilizer. Pathogens within soil may contaminate crops directly when heavy rain or water gun irrigation causes leaf splash.

The ability of the pathogen to survive in the environment will impact on the likelihood of crop contamination and pathogen viability at harvest and through to consumption. Initially, the pathogen must survive in the propagation environment until crops are planted out, or in organic wastes applied to the land. Survival times are often inconsistent and reflect the variability in propagation environments and organic waste treatments. Pathogenic bacteria and faecal indicator bacteria are known to survive in soil and manure for a longer period of time.

2.3.1.2 Post-harvest contamination

Post-harvest sources of contamination include human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dust, washing water, ice, transport vehicles, processing equipment, cutting, slicing, packaging, and shipping. Post harvesting processes, ranging from storage and rinsing to cutting, are also possible sources of contamination. Cut surfaces of leaves are a specific target for pathogenic bacteria such as *Salmonella.* The number of recent food-poisoning outbreaks have been linked to the consumption of fresh fruits and vegetables and that unhygienic product handling is implicated as the principal source of contamination. Poor hygienic conditions in processing increase the risk of contamination with foodborne pathogens.

The human hands, which come in contact with whole or cut produce, represent potential points of contamination throughout the total system of growing, harvesting, packing, processing, shipping, and preparing produce for consumption. Surface of vegetables may be contaminated by this organism through human handling and other environmental factors and can be able to survive for several days.

2.4 Microbial Load of Vegetables

Plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality (Aycicek *et al.*, 2004). These organisms reflect the exposure of the sample to any contamination and in general, the existence of favorable conditions for the multiplication of microorganisms. For various reasons, this parameter is useful to indicate if cleaning, disinfection and temperature control during industrial processing, transportation and storage, have been performed sufficiently (Tortora, 1995).

The number or population of bacteria present in raw vegetables among other things is greatly dependent on the amount of moisture present. Studies on cabbage and cauliflower (Lund, 1983) have shown that fresh vegetables at harvest can harbor as much as $10^5 - 10^7$ cells/gram of bacteria flora; a significant amount of which are fluorescent *Pseudomonas*. There is however relatively little quantitative information regarding the amount of wastage resulting from bacteria spoilage.

Microorganisms on the surface of freshly harvested fruit and vegetables include not only those of the normal flora but also those from soil and water and perhaps plant pathogens. Pathogens are derived from effluents from crude and treated sewage (Edel *et al.*, 1972) and if water thus polluted is used for irrigation, which is often the practice during long periods of drought, contamination of field crops may result. Many cases of illness caused by consumption of vegetables treated with surface water have been reported (Geldreich and Bordner, 1971).

Bacterial count on fresh vegetables to be processed upon arrival at the plant may range from $10^3 - 10^7$ per gram depending on the species and condition (Frazier and Westhoff, 1988). Bacteria associated with vegetables are said to include: *Pseudomonas, Alcaligenes, Erwinia, Xantomonas,* other Gram negative micrococci, *Bacillus*, lactic acid Bacteria and Coryneforms (Frazier and Westhoff, 1988). There is considerable variation in the number and types of microorganisms present on vegetables. The species, the amount of adhering dirt or soil, the location and the presence or absence of physical damage would all be significant variables.

2.5 Microorganisms associated with fresh vegetables

The microorganisms normally present on the surface of raw fruits and vegetables may consist of chance contaminants from the soil or dust. These include bacteria or fungi that have grown and colonized by utilizing nutrients exuded from plant tissues. Among the groups of bacteria commonly found on plant vegetation are those that test positive for coliforms or faecal coliforms, such as *Klebsiella* and *Enterobacter* (Zhao *et al.*, 2007). Microorganisms capable of causing human illness and others whose foodborne disease potential is uncertain, such as *Aeromonas hydrophila*, *Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella* spp. have been isolated in lettuce and salad vegetables (Francis *et al.*, 1999).

Foodborne bacterial pathogens commonly detected in fresh vegetables were coliform bacteria, *E. coli*, *Staphylococcus aureus* and *Salmonella* spp. (Tambekar, 2006). Coliforms are facultative anaerobic, Gram-negative, non-spore forming rods that ferment lactose with gas formation within 48 hours when grown in lactose broth at 35°C. They are commonly-used bacterial indicator of sanitary quality of foods and water and considered as an indicator of microbial pollution and they are common inhabitants of animal and human guts (Tortora, 1995). *Escherichia coli* is the species associated with faecal contamination and is naturally found in the intestines of humans and warm-blooded animals. The presence of these bacteria poses a serious threat to public health with outbreaks arising from food and water that has been contaminated by human or animal feces or sewage. *Staphylococcus aureus* is the third most common cause of confirmed food poisoning in the world (Acco *et al*., 2003) and the illness is due to the ingestion of preformed enterotoxins produced in foods.

2.6 Risk associated with the consumption of contaminated vegetables

Several outbreak of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables has occurred with increased frequency during the plant decade. Raw vegetables are widely consumed in the form of salad in most countries. Consumption of raw or slightly cooked vegetables can increase the risk of foodborne disease and increasingly recognized as vehicle for moulds dominate the microflora on raw vegetables (Beuchat, 1996).

Vegetables salads minimally processed containing raw vegetables, contaminated by pathogen that can be as vehicle of bacterial agent to human (Beuchat, 1996). As *listeria monocytogen, E. Coli* 0157:H7, and *Salmonella*, outbreak of enterohemorrhagic *E. Coli* 0157:H7 infection associated with eating contaminated vegetables as lettuce and other leaf crops have occurred with increasing frequency in recent years. Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilizer with contaminated soil or sewage.

2.7 Prevention and control of Microbial Contamination of Fresh Vegetables

The ability of public health agencies to identify through enhanced epidemiological and surveillance techniques, raw vegetables as probable sources of infections, the risk of illness associated with raw vegetables products can be reduced by removing or killing pathogenic microorganisms by washing or treating them with sanitizers. However, the hydrophilic cutin, diverse surface morphologies and abrasions in the epidermis of fruits and vegetables limits the efficacy of this treatment.

Vegetables are frequently consumed raw without being exposed to the processes that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce bacterial levels but cannot be relied upon to eliminate pathogens. However cleaning processes reduce the number of microorganisms. Fresh vegetables and fruits must be washed or treated specifically to minimize microbial load.

While a limited amount of contamination of vegetables will take place between harvesting and processing or consumption, gross contamination can be avoided. Boxes, lugs, baskets and other containers should be practically free of the growth of microorganisms, and some will need cleaning and sanitation between uses. Contamination from equipment at the processing plant can be reduced by adequate cleaning and sanitizing.

Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide good reductions in yield loss due to spoilage at all subsequent steps in the processing.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

A total of 30 samples comprising 10 each of three vegetable sample [cabbage (10), lettuce (10) and cucumber (10)] were purchased from ten (10) different retailers at Tudun-wada market. The samples were collected weekly for three weeks during which ten (10) samples per week was collected from different retailers. All the samples were collected polythene bags as sold and transported to the microbiology laboratory at Federal University Gusau, for analysis within an hour after collection. The polythene bags were opened under aseptic condition; by cleaning the surface of the working bench/table first with disinfectant and then a clean foil paper was spread on it on which the vegetables were placed, after being removed from the polythene bags. The analysis was then carried out immediately.

3.2 Preparation of Sample

Ten (10) grams of each vegetable sample was weighed using mettle weighing balance and then homogenized in 90ml of sterile distilled water to form the stock solution (homogenate) of the sample.

3.3 Media Preparation

The following commercially available dehydrated media were prepared in accordance with the manufacturer's instructions: Nutrient agar, MacConkey agar, motility medium, peptone water, Kovac's indole reagent, Methyl Red and Voges Proskauer (MR - VP) reagent, TSI (Triple sugar ion) agar, Simmon citrate and Gram staining reagents.

on the slide. A positive test result was indicated by bubbling and frothing signifying gas production while in the absence of these, it signifies a negative test result.

3.5.4 Indole Test

The isolates were grown in 5ml peptone water for 24 hours. After this, 3 drops of Kovac's indole reagent was added and shaken gently. A positive reaction was indicated by the development of a red colour in the reagent layer above the broth within one minute while in the negative reaction, the indole reagent would retain its yellow colour.

3.5.5 Citrate Test

The isolates were inoculated into Simmon citrate agar slant in a test tubes and incubated for 24 – 72 hours at 37°C the development of a deep blue colour would indicate a positive reaction while in the negative reaction, the Simmon citrate agar retained its green colour.

3.5.6 Methyl Red – Voges Proskauer (MR – VP) Test

The isolates were grown in 5ml of MR – VP broth and incubated for 48 – 72 hours at 37°C. After this 1ml of the broth was transferred into a sterile serological tube. To this small quantity 3 drops of methyl red would be added. A red colour on the addition of the indicator signified a positive methyl red test, a yellow colour signified a negative test. To the remaining broth in the original tubes, 5 drops of 40% potassium hydroxide (KOH) was added followed by 15 drops of alpha-napthol in ethanol. The tube was then shaken and placed in a sloping position. The development of red colour starting from the liquid air interfere within one hour indicated a positive Voges Proskauer test result. Absence of red colour indicated a negative result.

3.5.7 Test in Triple Sugar Iron Agar (TSI)

The isolates were inoculated by streaking the surface of the slant and stabbing the butt 3 times in the TSI medium. The cap of the tube was loosen and incubated at 37°C for 24 hours. Gas formation was determined by the appearance of one or several bubbles in the butt. Vigorous gas formation resulted in cracks in the butt or in some cases the butt was pushed from the bottom.

Hydrogen sulphide formation was determined by the blackening of the whole butt or a ring of blackening at the slant butt junction. Glucose fermentation was indicated by the butt becoming yellow, when no other sugar (i.e. lactose or/and sucrose) was fermented, the slant was red while the butt was yellow. This is referred to as Alkaline/Acid or (K/A) reaction. When gas is also formed it was indicated by the designation (K/AG). But when in addition to glucose fermentation, lactose or sucrose or both sugar were fermented in the medium, both the butt and the slant became yellow. The reaction is designated as A/A or A/G (if gas is formed).

3.5.8 Urease Test

The isolates were inoculated into urea agar slant in a test tubes and incubated for 24 hours at 37°C the development of a bright pink colour would indicate a positive reaction while in the negative reaction, the urea agar retained its yellow colour.

CHAPTER FOUR

RESULTS

The data in Table 1 shows the mean bacterial load and coliform index, followed by the standard deviations and range of the selected fresh vegetable food samples (lettuce, cabbage and cucumber respectively) analyzed, which were obtained from ten (10) different retailers.

The data in Table 1 also shows that cabbage has the highest mean bacterial count (1.38×10^8 cfu/g) followed by lettuce (1.16×10^8 cfu/g) then cucumber (1.07×10^8 cfu/g). The table also reveals that cabbage had the highest number of coliforms (1.15×10^8 cfu/g) followed by lettuce (9.89×10^7 cfu/g) and then cucumber (9.57×10^7 cfu/g).

Table 1: Total Bacterial and Coliform Counts of Vegetable Food Samples Analyzed

Sample	N	Total Bacterial Count		Total Coliform Count	
		Mean (SD)	Range	Mean (SD)	Range
Lettuce	10	$1.16x10^8$ $(7.42x10^7)$	$1.94x10^8$	$9.89x10^7$ $(4.55x10^7)$	$1.51x10^8$
Cabbage	10	$1.38x10^8$ $(6.6x10^7)$	$1.99x10^8$	$1.15x10^8$ $(5.35x10^7)$	$1.72x10^8$
Cucumber	10	$1.07x10^8$ $(5.38x10^7)$	$1.56x10^8$	$9.57x10^7$ $(5.29x10^7)$	$1.89x10^8$

N indicates number of samples analyzed. Mean counts are indicated without brackets, while the corresponding standard deviations are indicated in brackets followed by the range. Total bacterial and coliform counts are expressed as colony forming units per gram (cfu/g).

CHAPTER FIVE

DISCUSSION

The microorganisms present in vegetables are a direct reflection of the sanitary quality of the cultivation water, havesting, transportation, storage and processing of the produce (Beuchat, 1996). All the bacteria isolated in this study have previously been isolated from vegetables in other studies, both in Nigeria and elsewhere (Olayemi, 1997; Uzeh *et al.*, 2009; Abdullahi and Abdulkareem 2010).

This study revealed that the mean bacterial load was higher in cabbage (1.38×10^8 cfu/g) compared to lettuce (1.16×10^8 cfu/g) and cucumber (1.07×10^8 cfu/g). The data in Table 1, also reveals that cabbage had the highest number of coliform counts (1.15×10^8 cfu/g) followed by lettuce (9.89×10^7 cfu/g) and then cucumber (9.57×10^7 cfu/g) having the lowest bacterial load.

The microbial loads of vegetables evaluated in Table 1 were far higher than WHO standards of TBC 1.6x10 cfu/g and total coliform count 1x10 cfu/g (WHO, 1996) this result is in line with the findings of Abdulkareem (2004). According to Geldrich and Bordner (1971), high bacterial counts in vegetables (as obtained in this study) could be attributed to the unhygienic conditions and practice as well as the extent of exposure to dust under which they are displayed. Coliforms are indicator of unsanitary conditions, unhygienic practices during and after production and poor source of water used (Beuchat, 1996).

The high microbial contamination observed in the vegetables in this study may be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling. Bacteria on storage materials may transfer to produce and cross contamination between produce is probable particularly where produce are pre-washed with the same wash water by the vendor or processor. More importantly, bacteria on the produce may multiply over time depending on the storage conditions especially those that are phsychrotrophic (Abadias *et al.*, 2008).

Among the Genera of bacteria isolated in this study as shown in Table 2, *Escherichia coli*, *Bacillus* specie and *Staphylococcus* specie were predominant in all the 3 (three) vegetables analysed. The presence of these organisms in vegetables can be due to ecological and environmental influence since their survival in the atmosphere depends on a number of factor such as nature of microorganism, susceptibility to changes, resistance to new physical environment and their ability to form resistant spores. Godon (2007) reported that endospore of *Bacillus* are more resistant than vegetative cells to heat, drying, disinfectants and other destructive agents thus remaining viable for centuries, hence the high frequency of isolation of *Bacillus* specie from the analysed samples.

Also, the *Staphylococcus* specie isolated in this study could be attributed to poor hygienic, poor handling, poor storage conditions and also the manner in which the vegetables are sold that continually predisposes them to contamination (Geldrich and Border, 1971). The consumption of this bacteria (*Staphylococcus*) may cause health hazard because *Staphylococcus* species secrete enterotoxins which causes serious intoxication in man.

Enterobacter species are environmental contaminants. Their presence in vegetable indicates poor preparation and handling practices, cross contamination, unclean hands of the vendors and contact with sewage or contaminated water (Beuchat, 1996).

Klebsiella sp. isolated in some vegetables further highlights the need to safeguard the health of the consumers by proper washing and decontamination of these produce which are consumed without heat treatment.

Coliforms are indicators of feacal contamination by enteric bacteria. The high coliform count obtained in this study (i.e. average coliform count ranging from $3.10 \times 10^7 - 1.82 \times 10^8$ cfu/g, $3.80 \times 10^7 - 2.10 \times 10^8$ cfu/g and $2.20 \times 10^7 - 2.11 \times 10^8$ cfu/g for lettuce, cabbage and cucumber respectively) where in line with the findings of Abdullahi and Abdulkareem (2010) and this could be attributed to the effluent from sewage and run off water which was used for irrigation as its often practiced in this part of the country.

5.1 Conclusion

The study revealed high microbial load in the vegetables investigated (lettuce, cabbage and cumber), the study also shows that there is contamination in fresh vegetables sold in Tudun-wada market. The average counts for bacteria were above the maximum allowable limit in foods to be marked for consumption (1.6x10 cfu/g). Of the five bacterial species isolated (*Escherichia coli, Bacillus* sp., *Klebsiella* sp., *Enterobacter* sp. and *Staphylococcus* sp.) some occurred in three food samples, others occurred in two food samples.

5.2 Recommendations

i. Consumers should endeavor to thoroughly wash vegetables before consumption particularly when given to children so as to reduce the bacterial load on them and possible elimination of pathogenic microorganisms.

ii. Government should provide hygiene inspectors to inspect vegetables before they are being sold to consumers in other to limit consumer infection and enforce retailers adhere to stipulated hygiene rules and regulations as defaulters would be penalized.

iii. It is recommended that the storage and preservation of these vegetables should be given greater attention especially improved facilities at domestic level under controlled conditions.

iv. Cleanliness, proper handling and washing of vegetables with water before sale should be observed by retailers and highly stressed by the government.

v. It is recommended that vegetable retailers and vendors should be provided with adequate information as to the harmful and potentially hazardous effect of contaminated vegetables to consumers and should also be provided with relevant guidelines pertaining methods of preventing the aforementioned health problem.

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APPENDIX A

Biochemical Characterization of all Isolated Colonies from Lettuce, Cabbage and Cucumber Samples.

Isolates	Gram rxn	Morphology	Indole	MR	VP	Citrate	Catalase	Urease	Motility	Glu	Lac	Suc	Gas	H_2S	Genus/Specie
1	G+	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
2	G-	Rod	-	-	+	+	+	+	-	+	+	-	+	-	*Klebsiella* sp.
3	G+	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
4	G-	Rod	-	-	+	+	+	+	-	+	+	-	+	-	*Klebsiella* sp.
5	G+	Cocci	+	-	+	+	+	+	+	+	+	-	-	-	*Staphylococcus* sp.
6	G-	Rod	-	-	+	+	+	+	-	+	+	-	+	-	*Klebsiella* sp.
7	G+	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
8	G+	Rod	-	-	+	+	+	-	+	+	-	+	-	-	*Bacillus* sp.
9	G-	Rod	-	-	+	+	+	+	+	+	+	-	+	-	*Klebsiella* sp.
10	G-	Cocci	+	-	+	+	+	+	+	+	+	-	-	-	*Staphylococcus* sp.
11	G-	Rod	-	-	+	+	+	+	-	+	+	-	+	-	*Escherichia coli*
12	G-	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
13	G-	Rod	-	+	-	+	+	+	+	+	-	+	+	-	*Escherichia coli*
14	G+	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
15	G-	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
16	G-	Rod	-	+	-	+	+	+	+	+	+	+	+	-	*Escherichia coli*

17	G-	Rod	-	-	+	+	+	+	-	+	+	-	+	+	*Enterobacter* sp.
18	G-	Rod	-	+	-	+	+	+	+	+	+	+	+	-	*Escherichia coli*
19	G-	Cocci	+	-	+	+	+	+	-	+	+	-	-	-	*Staphylococcus* sp.
20	G+	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
21	G+	Cocci	+	-	+	+	+	+	-	+	+	-	-	-	*Staphylococcus* sp.
22	G-	Rod	-	-	+	+	+	+	-	+	+	-	+	+	*Enterobacter* sp.
23	G+	Cocci	+	-	+	+	+	+	+	+	+	-	+	+	*Staphylococcus* sp.
24	G-	Rod	-	+	-	+	+	+	+	+	+	+	+	-	*Escherichia coli*
25	G-	Cocci	+	-	+	+	+	+	-	+	+	+	-	-	*Staphylococcus* sp.
26	G-	Rod	-	+	-	+	+	+	+	+	+	+	+	-	*Escherichia coli*
27	G+	Cocci	+	-	+	+	+	+	-	+	+	-	-	-	*Staphylococcus* sp.
28	G+	Rod	-	-	+	+	+	+	+	+	-	+	-	-	*Bacillus* sp.
29	G-	Rod	-	+	-	+	+	+	+	+	+	+	+	-	*Escherichia coli*
30	G+	Rod	-	-	+	+	+	+	+	+	-	+	-	-	*Bacillus* sp.

Keys:

G+ = Gram positive

G- = Gram negative

+ = Positive test result

- = Negative test result

MR = Methyl Red

VP = Voges Proskauer

Suc = Sucrose

H_2S = Hydrogen sulphide

Glu = Glucose

Lac = Lactose

APPENDIX B

Bacterial Types Isolated from Different Vegetable Food Samples Analyzed

Bacterial Species	**Vegetable food samples**		
	Lettuce	**Cabbage**	**Cucumber**
Escherichia coli	-	+	+
Bacillus specie	+	+	+
Klebsiella specie	-	+	-
Enterobacter specie	+	-	+
Staphylococcus specie	+	+	+

Keys:

+ = Bacteria present

- = Bacteria not present