ABSTRACT

Fresh healthy carrot promote good health but harbour a wide range of contaminants. To assess the microbial quality of carrots sold in Ibadan, a total of fifteen samples were purchased from five vendors. Samples were analyzed to study the density of microorganisms by standard plate count.

Bacteria belonging to eight genera were identified. *Escherichia coli* (65%) was the most frequently isolated followed by *Staphylococcus aureus*, (57%) *Klebsiella spp, (*23%*)* were the least frequently isolated. Fungi such as *Aspergillus niger*, (38%) *Rhizopus spp*, (29%) and *Fusarium spp*,(16%) were also found associated with the samples and were identified based on their colonial and morphological characteristics. The effect of acetic acid concentration of 5% and exposure time of two minutes on the microbial load of the carrot samples were also assessed. However health education of the vendors and implementation of standard hygienic practices may reduce contamination of carrots for human consumption.

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CHAPTER ONE

1.0 INTRODUCTION

Vegetable is an edible component of plants or its part, intended for cooking or eating raw, (Vainio et al; 2003). In biological terms, "vegetable" designates members of the plant kingdom (Harri and Faber 2003). Vegetables may be raw or cooked, fresh, frozen, canned, or dried or dehydrated, and may be whole, cut-up, or mashed (USDA 2007). Vegetables are an extraordinary dietary source of nutrients, micronutrients, and vitamins, and are thus vital for health and well

being, vegetables are packed with soluble as well as insoluble dietary fiber known as non-starch polysaccharides (NSP) such as cellulose, mucilage, hemi-cellulose, gums, pectin, etc. These substances absorb excess water in the colon, retain a good amount of moisture in the fecal matter, and help its smooth passage out of the body, (Gupta 2008). Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin deficiencies and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2008). Based on their nutrient content, vegetables are organized into Five **(5**) sub groups which includes the dark green vegetables, starchy vegetables, red and orange vegetables, beans and peas, and other vegetables. Examples of Red and orange vegetables include carrots, pumpkin, red peppers, and tomatoes, (Gupta 2010).

Vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during post harvest processing. They therefore harbour a diverse range of microorganisms including plant and human pathogens (Nguyen-the and Carlin, 1994, Dunn et al; 1995, Carmo et al; 2004).

Carrot belongs to the sub group of red and orange vegetables, and which is also classified as a root vegetable, carrots belong to the Umbelliferae family, an extensive order of the herbaceous plants, and tagged a "native" of Britain, (James Main 1995).

Carrots are of great importance to man, carrots are often eaten cooked, many carrots are also consumed fresh, some are use as dish or stews while several others are poisonous.

Despite their nutritional and health benefits, out breaks of human infections associated with the consumption of fresh or minimally processed carrots have increased in recent years (Hedberg, 1996, Altekruse and Swerdlow, 1996, Beuchat, 1996, Beuchat, 2002). Enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns during food-related outbreaks (Buck et al; 2003). Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage (Beuchat, 2002). These increases in food borne infections may have resulted from increased consumption of contaminated carrots and vegetables,

Out breaks of food poisoning caused by biological hazards have been traced back to the consumption of carrots. In particular, *Escherichia coli* has been identified as the pathogen in several outbreaks involving carrots since 1990, (OMAF 2001).

Bacteriologically safe carrots are essential to maximize the health benefits promised by adequate consumption of these produce.

Carrots are contaminated by pathogens in different ways, through water, air, soil, pests, animals, cross-contamination and handlers. For instance contamination of carrots at harvest can occur when *Salmonella enterica* and *Escherichia coli* containing manures are applied to soils under conditions stimulating warm (daily average maximum temperature of >20 ◦C) summer conditions, (Carlin et al; 1994). (In contrast, the pathogens were not present in carrots harvested in soil to which non sterile manure had been applied and subjected to repeated freeze-thaw cycles (Natvig 2002), the storage of carrots at low temperatures may also favour the growth of pathogens such as *Yersinia pseudotuberculosis*, (Jalava et al; 1994).

Pathogenic microorganisms associated with carrots can cause severe outbreaks of food borne disease, they have been implicated in two outbreaks in England and Wales during 1992–2005 and in other two outbreaks in the United States during 1973–1997, (Jalava et al; 1994).

1.1 THE OBJECTIVES OF THE STUDY IS AS FOLLOWS

- To determine the microbial load of carrots sold in Ibadan, Oyo state, Nigeria
- Isolation and identification of microorganisms associated with retail carrots from five different vendors in Ibadan metropolis.
- Isolation of microorganisms associated with carrots to obtain a pure stock culture.
- To determine the effect of vinegar (acetic acid) on the microbial load of the carrots.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ORIGIN OF CARROT

Carrot has a somewhat obscure history, surrounded by doubt and enigma and it is difficult to pin down when domestication took place. The carrot can trace its ancestry back thousands of years, originally having been cultivated in central Asian and Middle Eastern countries, along with parts of Europe. These original carrots looked different from those that we are accustomed to today,

featuring red, purple, and yellow coloring rather than the bright orange that we've become accustomed to (Vavilov 1992).

Carrots were originally recorded as being cultivated in the present day Afghanistan about 1000 years ago, probably as a purple or yellow root, (Vavilov 1992). Carrot cultivation spread to Spain in the 1100s via the Middle East and North Africa, European settlers introduced the carrot to Colonial America in the 17th century.

The cultivated carrot is believed to originate from Afghanistan before the 900s, as this area is described as the primary centre of greatest carrot diversity (Mackevic 1992), Turkey being proposed as a secondary centre of origin (Banga 1993).

Cultivation of roots for consumption dates back to 600 A.D. when purple root types were grown in the area currently known as Afghanistan, (William et al; 2012).

Yellow types were eventually selected and produced in Syria and Iran in the ninth or tenth century. Carrots were introduced to China by the thirteenth century and cultivation spread from the Middle East to Italy, Spain and throughout Europe by the fourteenth century. Eventually, white and orange types were selected. Orange types, first grown in the Netherlands during the seventeenth century, were brought to North America by early settlers. The root was popular with Native Americans and production currently exists worldwide, (Darbie et al; 2012).

According to history, there exist varieties of carrots which are categorized by colour, they include the, orange carrots, purple carrots, yellow carrots, white carrots, and red carrots, (Kromhout et al; 2012).

Carrot is describe as a herbaceous root vegetable, the name "carrot" comes from the Greek word "karoton," whose first three letters (kar) are used to designate anything with a horn-like shape. (That horn-like shape, refers to the taproot of the carrot, (Doyle et al; 2012).

The most commonly eaten part of a carrot is a taproot, although the greens are sometimes eaten as well. It is a domesticated form of the wild carrot (*Daucus carota)*, native to Europe and south western Asia.

2.2 BOTANICAL CLASSIFICATION OF CARROT

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnolopsidia

Order: Apiales

Family: Apiaceae

Genus: *Daucus*

Specie: *Carota*

Source: New world encyclopedia (2012)

2.4 NUTRITIONAL VALUE OF CARROT

Carrots are nutritional heroes, they store a goldmine of nutrients, carrots nutritional profile includes information on a full array of nutrients, including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids and more, (Johrer et al; 2005). Carrot is an outstanding source of phyto nutrient, carrots actually contain a fascinating combination of phyto nutrients, including other carotenoids (especially alpha-carotene and lutein), hydroxycinnamic acids including (caffeic, coumaric, ferulic,) anthocyanins (in the case of purple and red carrots), and polyacetylenes (especially falcarinol and falcarindiol),(Zidorn et al; 2005). Carrots are an excellent source of vitamin A (in the form of carotenoids). In addition,

they are a very good source of biotin, vitamin K, dietary fiber, molybdenum, potassium, vitamin B6, and vitamin C. They are also a good source of manganese, niacin, vitamin B1, panthothenic acid, phosphorus, folate, copper, vitamin E, and vitamin B2. (Gilbert 2005).

2.5 IMPORTANCE OF CARROT

Carrots have numerous importance and benefits, that contributes and plays a vital role in the well-being of humans, carrots have numerous nutritional and dietary benefits upon human consumption.

Fresh carrots are essential components of the human diet and there is a considerable evidence of the health and nutritional benefits associated with the consumption of fresh carrots. In the USA, Canada, New Zealand and several countries like Nigeria, (Rennie et al; 2010).

All varieties of carrots contain valuable amounts of antioxidant nutrients. Included here are traditional antioxidants like vitamin C, as well as phyto nutrient antioxidants like beta-carotene, (Larsen et al; 2010).

Given their antioxidant richness, the cardiovascular system needs constant protection from antioxidant damage, (Wang et al; 2011).

Carrots contain both vitamin A and beta-carotene and are known to lower the risk of eye disease, hence the carrot's association with eye sight, vitamin A is essential for the formation of the chemical called retinal, whose presence in the retina is necessary for vision, carrots are high in Vitamin A, and a deficiency in this nutrient can cause some difficulty seeing in dim light, (Tang 2012). The anti-cancer benefits of carrot have been best researched in the area of colon cancer. Carrots contain a lot of beta carotene, which may help reduce a wide range of cancers. A substance called falcarinol that is found in carrots has been found to reduce the risk of cancer, (Matejkova et al; 2012).

2.6 MICROBIAL CONTAMINATION OF CARROT

Despite the nutritional and numerous health benefits, that are embedded in carrots, it is also pertinent to be aware that fresh and ready to eat carrot are high potential sources of microbial contamination, hence fresh carrot can be a vehicle for transmission of bacterial and fungal

pathogens capable of causing human illnesses, (Carlin et al; 1996). This is usually due to the series of essential agricultural procedures that can predispose carrot to pathogens, as well as other unhygienic practices of the handlers that can influence the invasion or colonization of pathogenic microorganisms, (Doyle 1999). Prevention of contamination of carrots with pathogenic microorganisms should be the goal of everyone involved in both the pre-harvest and post-harvest phases of delivering produce to the consumer. This is a very difficult task, since some pathogens are normally present in the soil and may therefore be present on the surface of carrots when they are harvested. Farm environments are not and cannot be aseptic. Reduction in the chances of contamination can be achieved, however, through appropriate agronomic practices, harvesting, processing, shipping, marketing and preparation, (Splittstoesser *et al;* 1990). The use of properly composted manure and properly treated irrigation and spray waters, as well as pathogen-free water for washing, will minimize the risk of contamination of carrot with microbial pathogens. Good hygienic practice during production and transport, including sanitizing of harvesting equipment and transport vehicles, as well as the application of good hygienic practice during processing and preparation are critical, (Zhao et al;1999*).* Elimination of animals and insects from processing, storage, marketing and food-service facilities should be a goal of anyone who handles raw produce. The highest level of hygiene must be practised by all handlers (including consumers) of carrot from the field to the table, if any degree of success is to be achieved in minimizing the risk of contamination, (Razzell, 1999).

The microorganisms normally present on the surface of raw carrot may consist of chance contaminants from the soil or dust, or bacteria or fungi that have grown and colonized by utilizing nutrients exuded from carrot tissues, Among the groups of bacteria commonly found on carrot vegetation are those that test positive for coli forms or faecal coli forms . e.g. *Klebsiella* and *Enterobacter* (Duncan 1997). Thus, the presence of coli forms or faecal coliforms on carrot does not necessarily provide index of faecal contamination.

Differences in microbial profiles result largely from unrelated factors such as resident micro flora in the soil, application of non-resident micro flora via animal manures, sewage or irrigation water, rainfall and atmospheric humidity.

It is therefore necessary to isolate and identify these microorganisms, and as well as profer possible control that can eliminate or reduce to minimal or tolerable level, this pathogenic microorganisms.

2.7 Pathogens of most concern

2.7.1 *Salmonella*

The antigenic scheme for classifying salmonellae recognizes more than 2300 serovars and, while all can be considered human pathogens, only about 200 are associated with human illness.

Animal husbandry practices used in the poultry, meat and fish industries, and the recycling of offal and inedible raw materials into animal feeds, has favoured the continued prominence of *Salmonella* in the global food chain (Aoust, 1997). There are reports of human salmonellosis linked to the consumption of carrots (Ries *et al;* 1990). Hygienic conditions during the production, harvesting, transport and distribution of carrot from some countries may not always meet minimum hygienic requirements, thus facilitating contamination on arrival in another country. Application of night soil, untreated sewage sludge or effluents, or irrigation water containing untreated sewage to fields and gardens can also result in contamination of carrot with *Salmonella* and other pathogens. Washing carrot with contaminated water and handling by infected workers, vendors and consumers in the marketplace helps the spread of pathogenic microorganisms, including *Salmonella*. *Salmonellae* have been isolated from many types of carrot (Beuchat, 1996b; Wells and Butterfield, 1997).

2.7.2 *Shigella*

Bacillary dysentery or shigellosis is caused by *Shigella*, of which there are four species namely *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* (Maurelli and Lampel, 1997). Most cases of shigellosis result from the ingestion of food or water contaminated with human faeces. Like *salmonellae* and other pathogens present in faeces, *Shigella* can contaminate raw carrot by several routes, including insects and the hands of persons who handle the produce, although shigellosis is more often transmitted from person to person.

Several large outbreaks of shigellosis have been attributed to the consumption of contaminated raw carrot.

2.7.3 *Escherichia coli*

Escherichia coli is common in the normal microflora of the intestinal tracts of humans and other warm-blooded animals. Strains that cause diarrhoeal illness are categorized into groups on the basis of virulence properties, mechanisms of pathogenicity, clinical syndromes and antigenic characteristics. The major groups are designated as enterotoxigenic, enterohaemorrhagic, enteropathogenic, enteroinvasive, diffuse-adhering and enteroaggregative (Doyle *et al*; 1997).

Carrot can become contaminated with one or more of these groups while in the field or during post-harvest handling. Sources and mechanisms of contamination are similar to those described for *Salmonella* and *Shigella.*

Enterotoxigenic *E. coli* is a cause of traveller's diarrhoea, an illness sometimes experienced when individuals visit countries with food and water hygiene standards different from their own.

Contaminated carrot are thought to be a common cause of traveller's diarrhoea, (Merson *et al*; 1976; Mintz, 1994) .Contamination of raw carrot with enterohaemorrhagic *E. coli* O157:H7 may occur when cattle, and perhaps other ruminants such as deer, inadvertently enter fields, or when improperly composted cow manure has been applied as a fertilizer. The potential for contamination may be enhanced when carrot have fallen from the plant to the ground and are then picked and placed into the handling and processing chain. Workers on farms and in packing houses may also be a source of *E. coli* O157:H7, (Nathan, 1997).

2.7.4 *Staphylococcus aureus*

Staphylococcus aureus is known to be carried in the nasal passages of healthy food handlers and has been detected on raw produce such as carrots (Abdelnoor *et al*; 1993). However, enterotoxigenic *Staphylococccus aureus* does not compete well with other normally present on raw carrot, so spoilage caused by non pathogenic micro flora would probably precede the development of the high populations of this pathogen that would be needed for production of staphylococcal enterotoxin, (Solomon *et al*; 1996).

2.8 SOURCES OF MICROBIAL CONTAMINATION IN CARROT

Contamination of carrots may take place at all stages during production, harvesting, sorting, packing, and transport and handling. Other possible sources of microorganisms include soil, faeces of both human and animal origin.

Microorganisms include: Fungi, bacteria, protozoans and viruses.

2.8.1 Source of contamination in carrot during production and harvest

Water of inadequate quality has the potential to be a direct source of contamination and a vehicle for spreading localized contamination in the field, or transportation environments. Water use in production involves numerous field operations including irrigation, applications of pesticides and post-harvest uses include produce rinsing, cooling, and washing.

Water can be a carrier of many microorganisms including pathogenic strains of *Escherichia coli*, *Salmonella spp*, *Shigella spp*. Small amounts of contamination with some of these organisms can result in food borne illness.

Hedburg et al; 1996 attributed two outbreaks, in 1990 and 1993, involving at least 300 cases in four countries attributed to *Salmonella* species, were linked to consumption of fresh carrot, the outbreaks were traced back to a single packing facility where a water-bath appeared to be the likely source of contamination, (Satta 2000).

During harvest or post harvest handling, when the skin of the carrot surface is punctured, scraped or sliced, microorganisms, such as *Listeria monocytogenes*, *Clostridium botulinum*, *Enterobacter, Pseudomonas aeruginosa,* and *Bacillus cereus* may begin to grow, (Chang et al; 2003). Hidemi et al; 2004 carried out a research on microbial evaluation of carrot and attributed the microbial contamination of the carrots to improper harvesting procedures.

The significance of contamination of carrot during pre harvest or processing is greatly influenced by the pathogens infectious dose and its fate during storage. With pathogens that have a low infectious dose, such as *Escherichia coli 0157:H7*, their prescence is unacceptable and contaminated carrot should not be distributed regardless of the pathogens fate, (Bradbury 2009). With pathogens whose infectious dose is high, such as *Bacillus cereus*, distribution of carrot contaminated with low populations may not be harmful unless storage conditions contribute to the pathogens growth, (Clayton 2008).

In general, factors that have been demonstrated to impact the fate of enteric pathogens on carrot include storage, temperature, relative humidity, atmosphere gas composition, nutrient availability, and prescence of competitive bacteria or antimicrobial compounds, (Ammar 1993).

2.8.2 Source of contamination of carrot during storage

Scheil et al; 1998 attributed poor storage associated with inadequate packaging of carrots which resulted in the contamination of mould growth, such as *Fusarium spp, Aspergillus niger,*

Penicillium spp, and *Rhizopus spp*, or in extreme cases can result in the production of mycotoxins.

He attributed *Salmonella* isolated from stored carrots, to the animal excreta found in the storage premises where carrot were stacked.

Chaturvedi et al; 2013 explained that improper storage resulted in the contamination of carrot, that caused deleterious mycotoxicosos resulting in the death of over twenty people in India in 1995.

2.8.3 Source of contamination in carrot during handling

Haris et al; 2008 explained that handling of carrot by infected field workers, cross contamination are few of the ways that transmit pathogenic microorganisms to carrots.

Data from the CDC (Centre for Disease Control) food borne outbreak surveillance system shows that from 1988 to 1998, the two most commonly reported microorganisms associated with fresh carrot food borne illnesses were *Salmonella spp*, and *Escherichia coli 0157:H7* with 45% and 38% of carrots linked outbreaks, (Sapers, 2009).

2.9 JUSTIFICATION OF STUDY

Experimental studies have demonstrated the potential of contamination in carrot to be contaminated during pre-harvest, post-harvest and storage, as well as the improper handling by field worker, retailer, which may result into contamination by pathogenic microorganisms such as *Escherichia coli* and *Salmonella spp*, hence the need for an assessment of the microbial safety of carrot, by the isolation and identification of microorganisms associated with carrot sold in

Ibadan metropolis, and also experiment the effect of acetic acid (vinegar) wash on the microbial load.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Equipment

Light microscope, incubator, inoculating needle, autoclave, beam balance, oven, spirit lamp, lamina chamber, electronic weighing balance, water bath.

3.2 Glass ware

Petri dishes, microscope slides, cover slips, 1000 ml measuring cylinder, 1000 ml conical flask, 1000 ml beaker and stirrer, test tubes.

3.3 Reagents and Chemicals

Distilled water, acetic acid, gram stain, lacto-phenol blue stain, ethanol

3.4 Media

Nutrient Agar**,** Potato Dextrose Agar**,** Eosin Methylene Blue Agar (EMB)**,** Salmonella Shigella Agar (SSA) and Mac-conkey Agar

3.5 Sample

Fresh healthy carrots

3.6 Materials

Aluminium foil, cotton wool, needle and syringe, mc-cartney bottles,

3.7 Collection of samples

Random sampling procedure was adopted to collect the sample materials, a total of fifteen (15) healthy carrot samples were collected from five different markets in Ibadan Bodija (Ibadan North Local Government Area) Oje (Ibadan North West Local Government Area) Gbagi, (Egbeda Local Government) Monatan, (Lagelu Local Government Area) and Challenge, (Oluyole Local Government Area). and brought into the laboratory in sterile polythene bags.

3.8 Sterilization of materials

All the glass wares were washed with detergent then rinsed with water. They were drained by arranging them on a rack, and wrapped with aluminum foil, and then sterilized in an autoclave for 15 minutes at 121^0C with a pressure of 15psi . Inoculating needles was sterilized by dipping it in 95% ethanol and then flamed before and after each use. The carrots were rinsed with tap water followed by sterile distilled water.

3.9 Preparation of media

3.9.1 Preparation of Potato Dextrose Agar

According to the manufacturers' instructions, 39g of potato dextrose agar powder was weighed using an electronic weighing balance and poured into 1000ml conical flask. It is swirled to mix and then homogenized in a shaking water bath at 100^0C for 20 minutes. The mouth of the conical flask containing the homogenized agar was plugged with a non-absorbent cotton wool, the conical flask was wrapped with aluminum foil and then sterilized in an autoclave for 15 minutes at 121^0C with a pressure of 15psi, after autoclaving was allowed to cool to about 30^0C, it is then swirled slightly before pouring aseptically into sterile petri dish and the agar solidified thereafter.

3.9.2 Preparation of Nutrient Agar

According to the manufacturers' instructions, 28g of nutrient agar powder was weighed using an electronic weighing balance and poured into 1000ml conical flask. It is swirled to mix and then homogenized in a shaking water bath at 100^0C for 20 minutes. The mouth of the conical flask containing the homogenized agar was plugged with a non-absorbent cotton wool, the conical flask was wrapped with aluminum foil and then sterilized in an autoclave for 15 minutes at 121^0C with a pressure of 15psi, it was allowed to cool to about 50^0C, it is then swirled slightly before pouring aseptically into sterile culture petri dish and the agar solidified thereafter.

3.9.3 Preparation of Eosin Methylene Blue Agar

According to the manufacturers' instructions, 37.5g of Eosin Methylene Blue agar powder was weighed using an electronic weighing balance and poured into 1000ml conical flask. It is swirled to mix and then homogenized in a shaking water bath at 100^0C for 20 minutes. The mouth of the conical flask containing the homogenized agar was plugged with a non-absorbent cotton wool, the conical flask was wrapped with aluminum foil and then sterilized in an autoclave for 15minutes at 121^0C with a pressure of 15psi, it was allowed to cool to about 50^0C, it is then swirled slightly before pouring aseptically into sterile culture petri dish and the agar solidified thereafter.

3.9.4 Preparation of Salmonella Shigella Agar

According to the manufacturers' instructions, 63g of Salmonella Shigella agar powder was weighed using an electronic weighing balance and poured into 1000ml conical flask. It is swirled to mix and then homogenized in a shaking water bath at 100^0C for 20minutes. The mouth of the conical flask containing the homogenized agar was plugged with a non-absorbent cotton wool, the conical flask was wrapped with aluminum foil and then sterilized in an autoclave for 15minutes at 121^0C with a pressure of 15psi, it was allowed to cool to about 50^0C, it is then swirled slightly before pouring aseptically into sterile culture petri dish and the agar solidified thereafter.

3.9.5 Preparation of Mac-Conkey Agar

According to the manufacturers' instructions, 48.5g of Mac-conkey agar powder was weighed using an electronic weighing balance and dispersed into 1000ml conical flask. It is swirled to mix and then homogenized in a shaking water bath at 100^0C for 20minutes. The mouth of the conical flask containing the homogenized agar was plugged with a non-absorbent cotton wool, the conical flask was wrapped with aluminum foil and then sterilized in an autoclave for 15minutes at 121^0C with a pressure of 15psi, it was allowed to cool to about 50^0C, it is then swirled slightly before pouring aseptically into sterile culture petri dish and the agar solidified thereafter.

3.10 Serial dilution

Using separate sterile pipettes, 1ml of the inoculum suspension was withdrawn for the serial dilution, and thoroughly mixed, then a ten fold dilution was carried out, and three dilution factors were selected, this procedure is used to identify the number of viable microorganisms in a fixed amount of a liquid, serial dilution involves repeatedly mixing known amounts of source culture with (sterilized) liquid. 1 ml of the original suspension + 9 ml sterile water - 1:10 dilution, 1 ml of B + 9 ml sterile water = 1:10^3, 1 ml of C + sterile wated = 1:10^4, 1 ml of D + 9 ml sterile water=1:$10^{5.}$ One (1) ml that was added to 9 ml gave a 10-fold dilution, then fixed amounts of this dilution series are mixed with an appropriate agar and incubated, then different numbers of colonies were obtained.

3.11 Membrane Filter technique

The fresh carrots samples were filtered in a membrane filter through a filter paper of pore size (0.45 micro metre), after which the saturated filter paper was removed and inoculated into the prepared sterile culture plates, and incubated.

3.12 Preparation of slant culture

Pure cultures from culture plates were introduced into already prepared slant. This was done aseptically by introducing cultures with the aid of inoculating needle and incubated at the right temperature of 30°C for 72 hours to obtain stock cultures.

3.13 Methods of isolation

The healthy carrots sample were cut , 25g of each were weighed and washed with 100 ml of sterile distilled water, from which a stock was prepared and serial dilution were made to a power of five, three of the dilution 10^3,10^4,10^5 were pour plated at 1 ml per culture plate using Nutrient agar, Potato Dextrose agar, Mac-Conkey agar, Salmonella Shigella agar, and Eosin Methylene Blue agar, in triplicate then incubated at 37°C for 24-48 hours. The bacterial and fungal colonies on plate were observed daily and counted, to obtain the total number of bacteria and fungal culture.

3.14 Methods of identification

The colonial and cellular characteristics of individual colony of pure culture were examined. The colonial morphology of the pure culture of each fungus and bacterium was determined. Microscopic examination of each pure fungal and bacterial isolates was also carried out using ×10, ×40, ×100 lens objectives. The colonial characteristics determined includes colour and appearance of the colony on the surface agar. After then temporary slides of each bacterial and fungal colony were prepared to be able to examine the morphological structures of each colony under binocular microscope. Biochemical tests such as catalase, coagulase, indole, citrate and starch hydrolysis test were also carried out.

3.15 Lacto phenol cotton blue stain

Procedure

A drop of 70% alcohol was added to a microscope slide, then the specimen or material was immersed in the drop of alcohol. Two drops of the lacto phenol or cotton blue was added to the mountant or stain before the alcohol dries out, the cover slip was placed at the edge, and lower gently, avoiding air bubbles. The preparation was viewed under a microscope.

3.16 Gram stain

Procedure

A sterile microscopic slide of containing the cell sample to be stained was prepared, and heat fixed, by carefully passing the slide with a drop or small piece of sample on it through a bunsen burner three times, then the primary stain (crystal violet) was added to the sample or slide. The slide was then rinsed with a gentle stream of water for a maximum of five seconds to remove the unbound crystal violet, after which, Gram's iodine was added for one minute, this serve as a mordant, or an agent that fixes the crystal violet to the bacterial cell wall, the sample or slide was rinsed with acetone or alcohol for three seconds and rinsed again with a gentle stream of water. The alcohol added is used to decolourize the sample, secondary stain, Safranin was added to the slide, and washed again with a gentle stream of water for a maximum of five seconds. The Gram positive bacteria retained the primary stain (crystal violet) and turned purple in colour, while the Gram negative bacteria appeared red or pinkish when viewed under a microscope.

3.17 BIOCHEMICAL TESTS

3.17.1 Indole test .

Procedure

The tryptophan broth was inoculated with a 24 hours pure culture and Incubated at 37°C for 24-28 hours in ambient air, after which 0.5 ml of Kovac's reagent was added to the broth culture.

3.17.2 Citrate test

Procedure

Simmon's citrate agar slants were prepared and inoculated with a 24 hours pure culture using aseptic technique and one other agar slant was left un inoculated (this was used as a negative control), and incubated at an appropriate temperature for a period of 24 - 48 hours, citrate positive slants were intense prussian blue in colour.

3.17.3 Coagulase test

Procedure

A clean grease free and dry glass slide was used and a drop of plasma and distilled water or normal saline was added as a negative control, a 24 hours pure culture being tested was added aseptically in each drop, the slide was gently swirled for 5 to 10 seconds, to check for any clumping or coagulation.

3.17.4 Urease test

Procedure

The surface of the urea agar slant was inoculated with a 24 hours pure culture and incubated for

48 hours, slants that changed from light orange to magenta were recorded to be positive to urease production, while some of the slant remained light orange, which indicated a negative result.

3.17.5 Catalase test

Procedure

A small amount of a 24 hours pure culture was added to a surface of a clean, dry glass slide using a loop or sterile wooden stick. A drop of 3% H_2O_2 (hydrogen peroxide) was added on to the slide and mixed. A positive result within 5-10 seconds produced air bubbles, while those that were negative showed no bubble.

3.17.6 Methyl Red and Voges-Proskauer (MR-VP)

Procedure

The isolated colony were inoculated aseptically into the MR-VP tubes, an 18-24 hour culture was used, and the MR-VP tubes were incubated, at 35-37°C for 48 hours. After incubation, a sterile pipette was used to remove 1-2 ml aliquots and placed into two small tubes, one tube was for the methyl red test and the other was for the Voges-Proskauer test, and 5 drops of methyl red was added to one tube, in which the result was read immediately. For the Voges-Proskauer test 15 drops of Voges-Proskauer A reagent, was added, and thoroughly mixed then another 5 drops of Voges-Proskauer B was added to the tube and mixed, the results were read within 5-15 minutes.

3.17.7 Starch hydrolysis test

Procedure

An Inoculated cultured plate of a 24 hours pure culture organism to be tested was Incubated at an optimum temperature for at least 48 hours, and the culture plates was flooded with iodine, and observed for any colour change, a blue colour was seen and indicated no hydrolyis, while a clear zone that was seen indicated hydrolysis.

3.18 Control experiment

For the control experiment, 5% acetic acid concentration was prepared, 1000 ml of 5% acetic acid was added to 25g of fresh healthy carrots, and allowed to stay for two minutes, and from each serial dilution was made, and pour plated at 1 ml per plate into triplicate, then incubated at 37°C for 24-48 hours. The bacterial and fungal colonies on plate were observed daily and counted, to obtain the total number of bacteria and fungal culture.

CHAPTER FOUR

4.0 RESULTS

Based on morphological, and colonial characteristics the following fungi namely *Rhizopus spp, Mucor spp, Fusarium spp, Yeast, Aspergillus niger, and Penicillium spp* were isolated from the

carrot samples. Table 4.1 shows the frequency and the percentage occurrence of fungi. However *Aspergillus niger* was isolated from all the locations.

Table 4.1

Frequency and percentage occurrence of fungi isolated from the carrot samples in November 2013.

	Frequency (Percentage occurrence)	**Location**				
Fungi isolates	**No (%)**	**Gbagi**	**Bodija**	**Monatan**	**Oje**	**Challenge**
Rhizopus spp	2(16.7)	-	+	-	+	-
Fusarium spp	1(8.3)	+	-	-	-	-
Yeast	2(16.7)	-	+	+	-	-
Aspergillus niger	5(41.7)	+	+	+	+	+
Penicillium spp	2(16.7)	+	-	-	-	+
Total	12 (100%)					

Carrot samples obtained from Gbagi had a total number of three fungi namely *Fusarium spp*, (8.3%) *Aspergillus niger,* (41.7%) and *Penicillium spp*, (16.7%). Carrot samples obtained from Bodija had a total number of three fungi namely *Rhizopus spp*, (16.7%), *Yeast* (16.7%) and *Aspergillus niger* (41.7%). Carrot samples obtained from Monatan had a total number of two fungi namely Yeast (16.7%) and *Aspergillus niger* (41.7%), Carrot samples obtained from Oje had a total number of two fungi namely *Rhizopus spp*, (16.7%) and *Aspergillus niger*, (41.7%), Carrot samples obtained from Challenge also had a total of two fungi namely *Aspergillus niger*, (41.7%) and *Penicillium spp* (16.7%).

During the second sampling, based on morphological, and colonial characteristics the following fungi namely, namely *Rhizopus spp, Mucor spp, Yeast, Aspergillus niger, and Penicillium spp,* were encountered in the second sampling. Table 4.2 shows the frequency and the percentage occurrence of the fungi isolates.

Table 4.2

Frequency and percentage occurrence of fungi isolated from the carrot samples in March 2014

	Frequency (Percentage occurrence)	Locations				
Fungi isolates	**No(%)**	**Gbagi**	**Bodija**	**Monatan**	**Oje**	**Challenge**
Rhizpous spp	3 (20%)	+	-	-	+	+
Yeast	3(20%)	-	+	-	+	+
Aspergillus niger	3(20%)	+	+	+	-	-
Penicillium spp	2(13%)	+	-	-	-	+
Mucor spp	4(26.7%)	-	+	+	+	+
Total	15(100%)					

Carrot samples obtained from Gbagi had a total number of three fungi namely *Rhizopus spp*, (20%), *Aspergillus niger,* (20%), and *Penicillium spp,* (13%). Carrot samples obtained from Bodija had a total number of three fungi namely Yeast (20%), *Aspergillus niger*, (20%), and *Mucor spp*, (26.7%). Carrot samples obtained from Monatan had a total number of two fungi namely *Aspergillus niger* (20%) and *Mucor spp,* (26.7%). Carrot samples obtained from Oje had a total number of three fungi namely *Rhizopus spp*,(20%), *Yeast*,(20%) and *Mucor spp,* (26.7%). Carrot samples obtained from Challenge also had a total of three fungi namely *Rhizopus spp*, (20%), *Yeast*(13%), and *Penicillium spp* (26.4%).

During the third sampling, based on morphological, and colonial characteristics, the following fungi namely *Rhizopus spp*, *Fusarium spp*, *Yeast*, and *Aspergillus niger*, were encountered. Table 4.3 shows the frequency and the percentage occurrence of the fungi isolates. However *Rhizopus spp* was isolated from carrot samples obtained from all the locations.

Table 4.3

Frequency and percentage occurrence of fungi isolated from the carrot samples in May 2014.

Frequency	Locations

(Percentage occurrence)

Fungi isolates	No(%)	Gbagi	Bodija	Monatan	Oje	Challenge
Rhizpous spp	5(38.47)	+	+	+	+	+
Fusarium spp	3(23.0)	-	+	+	+	-
Yeast	3(23.0)	+	+	-	+	-
Aspergillus niger	2(15.38%)	-	-	+	-	+
Total	13(100%)					

This table shows the microbial load in (CFU/ml) of the carrot samples obtained from different vendors, the microbial load ranged from (317.3 x 10^3 to 127 x10^5) for Bodija, and (235 x10^3 to 86x10^5) for Gbagi, and (422×10^3 to 65 x10^5) for Monatan, and (517×10^3 to 97 x10^5) for Challenge and (282×10^3 to 61×10^5) for Oje.

TABLE 4.4 This table shows the microbial load in (CFU/ml) of the carrot samples

	Locations									
Sampling No.	**Bodija**		**Gbagi**		**Monatan**		**Challenge**		**Oje**	
S/N	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)
1st	10^{-3} 10^{-4} 10^{-5}	317×10^{3} 362×10^{4} $198\ x10^{5}$	10^{-4} 10^{-4} 10^{-5}	$168x10^{3}$ $73x10^{4}$ $181x10^{5}$	10^{-3} 10^{-4} 10^{-5}	422×10^{3} 285×10^{4} 76×10^{5}	$1o^{-3}$ 10^{-4} 10^{-5}	517×10^{3} 361×10^{4} 225×10^{5}	$1o^{-3}$ 10^{-4} 10^{-5}	282×10^{3} 149×10^{4} 48×10^{5}
2nd	10^{-3} 10^{-4} 10^{-5}	$252\ x\ 10^{3}$ $106\ x\ 10^{4}$ $167x\ 10^{3}$	10^{-4} 10^{-4} 10^{-5}	$206\ x10^{3}$ $167\ x10^{4}$ $179\ x10^{5}$	10^{-3} 10^{-4} 10^{-5}	542×10^{3} 236×10^{4} 212×10^{5}	$1o^{-3}$ 10^{-4} 10^{-5}	435×10^{3} 413×10^{4} 259×10^{5}	$1o^{-3}$ 10^{-4} 10^{-5}	176×10^{3} 84×10^{4} 61×10^{5}
3rd	10^{-3} 10^{-4} 10^{-5}	$387\ x\ 10^{3}$ $218\ x\ 10^{4}$ $127\ x10^{5}$	10^{-4} 10^{-4} 10^{-5}	$235\ x10^{3}$ $226\ x10^{4}$ $86x10^{5}$	10^{-3} 10^{-4} 10^{-5}	235×10^{3} 226×10^{4} $65\ x10^{5}$	$1o^{-3}$ 10^{-4} 10^{-5}	156×10^{3} 138×10^{4} $97\ x10^{5}$	$1o^{-3}$ 10^{-4} 10^{-5}	232×10^{3} 289×10^{4} $186\ x10^{5}$

Based on cultural, morphological and biochemical characteristics, Seven bacteria namely *Escherichia coli, Klebsiella spp, Salmonella spp, Enterobacter spp, Staphylococcus aureus*, *Pseudomonas aeruginosa and Proteus,* were isolated from the carrot samples. Table 4.1 shows the frequency and the percentage occurrence of the bacteria isolates. However *Escherichia coli* was isolated from all the locations.

Table 4.5

Frequency and percentage occurrence of bacteria isolated from the carrot samples in Nov.2013

	Frequency	Locations				
Bacteria isolates	**No (%) Gbagi**	**Bodija**	**Monatan**	**Oje**	**Challenge**	
(Percentage occurence)						
Escherichia coli	5(22.72)	+	+	+	+	+
Klebsiella spp	4(18.18)	+	-	+	+	+
Salmonella spp	4(18.18)	-	-	-	+	+
Enterobacter spp	2(9.0)	-	+	-	+	-
Staphylococcus aureus	3(14.2)	+	+	-	+	-
Proteus	3(13)	-	+	+	+	-
Pseudomanas aeruginosa	1(4)	-	+	-	-	-
Total	22(100%)					

Carrot samples obtained from Gbagi had a total number of two bacteria namely *Escherichia coli* (22%), *Klebsiella spp* (18%), and *Staphyloccus aureus* (14.2%). Carrot samples obtained from Bodija had a total number of five bacteria namely *Escherichia coli* (22%), *Enterobacter spp* (9.0%), *Staphyloccus aureus* (14.2%), *Proteus* (13%), and *Pseudomonas aeruginosa* (4%). Carrot samples obtained from Monatan had a total number of three bacteria namely *Escherichia coli* (22%), *Klebsiella spp* (18.18%) and Proteus (13%). Carrot samples obtained from Oje had a total number of six bacteria namely *Escherichia coli* (22%), *Klebsiella spp* (18.18%) Proteus (13%) and Staphyloccus aureus (14.2%), *Enterobacter spp* (9.0%) and *Salmonella* (18.18%). Carrot samples obtained from Challenge also had a total of three bacteria namely *Enterobacter spp* (9.0%), *Salmonella spp* (18.18%), and *Klebsiella spp* (18.18%).

During the second sampling, based on cultural, morphological and biochemical characteristics, five bacteria namely *Escherichia coli*, *Klebsiella spp*, *Salmonella spp*, *Enterobacter spp*, and *Staphylococcus aureus*, were encountered.

TABLE 4.6

Frequency and percentage occurrence of fungi isolated from the carrot samples in March 2014

Frequency (Percentage occurrence)		Locations				
Bacteria isolates	**No (%)**	**Gbagi**	**Bodija**	**Monatan**	**Oje**	**Challenge**
Escherichia coli	3(18.75)	-	+	-	+	+
Klebsiella spp	4(25%)	-	+	+	+	+
Salmonella spp	4(25%)	+	+	+	-	+
Enterobacter spp	2(12.5)	-	+	-	+	-
Staphylococcus aureus	3(18.75)	+	-	+	-	+
Total	16(100%)					

Carrot samples obtained from Gbagi had a total number of two bacteria namely *Salmonella spp* (18.75%), and *Staphyloccus aureus* (18.75%). Carrot samples obtained from Bodija had a total number of four bacteria namely *Escherichia coli (*18.75%), *Klebsiella spp* (25%), *Salmonella spp* (25%), and *Enterobacter spp* (12.5%). Carrot samples obtained from Monatan had a total number of three

bacteria namely *Klebsiella spp* (25%), *Salmonella spp* (25%), and *Staphylococcus aureus* (18.75%). Carrot samples obtained from Oje had a total number of three bacteria namely *Escherichia coli* (18.75%), *klebsiella spp* (25%), and *Enterobacter spp* (12.5%). Carrot samples obtained from Challenge also had a total of four bacteria namely *Escherichia coli* (18.75%), *Klebsiella spp* (25%), *Salmonella spp* (25%), and *Staphyloccus aureus* (18.75%).

Based on cultural, morphological and biochemical characteristics, during the third sampling, six bacteria namely *Escherichia coli*, *Klebsiella spp, Salmonella spp, Enterobacter spp,* and *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were also encountered. Table 4.7 shows the frequency and the percentage occurrence of the bacteria isolates.

TABLE 4.7 Shows the frequency and the percentage occurrence of the bacteria isolates.

Frequency (Percentage occurrence)	**Locations**

Bacteria isolates	No (%)	Gbagi	Bodija	Monatan	Oje	Challenge
Escherichia coli	2(13.33)	-	+	-	+	-
Klebsiella spp	1(6)	-	+	-	-	-
Salmonella spp	4(26.67)	+	-	+	+	+
Enterobacter spp	2(13.33)	-	+	+	-	-
Staphylococcus aureus	3(20)	+	+	-	+	-
Pseudomonas aeruginosa	2(13.33)	+	-	+	-	-
Total	15 (100%)					

Carrot samples obtained from Gbagi had a total number of three bacteria namely *Salmonella spp* (26%), *Staphylococcus aureus* (20%) and *Pseudomonas aeruginosa* (13.33%).

Carrot samples obtained from Bodija had a total number of four bacteria namely *Escherichia coli* (13.33%), *Klebsiella spp* (6%), *Enterobacter spp* (13.33%), and *Staphyloccus aureus* (20%). Carrot samples obtained from Monatan had a total number of three bacteria namely *Salmonella spp* (26.67%), *Enterobacter spp* (13%), *Pseudomonas aeruginosa* (13%). Carrot samples obtained from Oje had a total number of three bacteria namely *Escherichia coli* (23%), *Staphylococcus aureus* (26%), and *Salmonella spp* (20%). While carrot samples obtained from Challenge only had one bacteria present namely *Salmonella spp* with an occurrence of (26.67%).

Table 4.8 Microbial load in (CFU/ml) of the carrot samples before treatment with 5% acetic acid.

This table shows the microbial load in (CFU/ml) of the carrot samples obtained from different vendors, before treatment with 5% acetic acid (vinegar) concentration.

	Locations									
Sampling No.	**Bodija**		**Gbagi**		**Monatan**		**Challenge**		**Oje**	
S/N	Dilution factors	Microbial Count	Dilution factors	Microbial Count	Dilution factors	Microbial Count	Dilution factors	Microbial Count	Dilution factors	Microbial Count

		(CFU/ml)		(CFU/ml)		(CFU/ml)		(CFU/ml)		(CFU/ml)
1st	10^{-3}	317×10^{3}	10^{-4}	$168x10^{3}$	10^{-3}	422×10^{3}	$1o^{-3}$	517×10^{3}	$1o^{-3}$	282×10^{3}
	10^{-4}	362×10^{4}	10^{-4}	$73x10^{4}$	10^{-4}	285×10^{4}	10^{-4}	361×10^{4}	10^{-4}	149×10^{4}
	10^{-5}	$198\ x10^{5}$	10^{-5}	$181x10^{5}$	10^{-5}	76×10^{5}	10^{-5}	225×10^{5}	10^{-5}	48×10^{5}

Table 4.9 Effect of acetic acid treatment of 5% concentration

This table shows the effect of acetic acid treatment (vinegar) of 5% concentration on the microbial load of carrot samples

	Locations									
Sampling No.	**Bodija**		**Gbagi**		**Monatan**		**Challenge**		**Oje**	
S/N	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)	Dilution factors	Microbial Count (CFU/ml)

1st	10^{-3} 10^{-4} 10^{-5}	120×10^{3} 251×10^{4} $96\ x10^{5}$	10^{-4} 10^{-4} 10^{-5}	$52x10^{3}$ $21x10^{4}$ $181x10^{5}$	10^{-3} 10^{-4} 10^{-5}	124×10^{3} 65×10^{4} NIL	$1o^{-3}$ 10^{-4} 10^{-5}	NIL NIL 34×10^{5}	$1o^{-3}$ 10^{-4} 10^{-5}	NIL 75×10^{4} 21×10^{5}

NIL: Indicates absecnce of microbial colony

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The results of this work shows that fungi such as, *Aspergillus niger, Rhizopus spp, Yeast, Fusarium spp,* and *Mucor spp,* were found to be associated with the carrot, while bacteria such

as *Escherichia coli, Klebsiella spp, Staphylococcus aureus, Shigella spp, Proteus spp,* and *Enterobacter* were also found to be associated with the carrots.

In this study, the microbial load was determined on the various culture media that were used, for instance Nutrient agar, and Potato Dextrose Agar by spread plate count method (Khan *et al*., 1992). Among the carrots analyzed, samples sourced from Bodija market in Ibadan, Oyo state had the highest microbial load.

The presence of *Enterobacter spp, Klebsiella spp, Escherichia coli,* and S*taphylococcus aureus* was observed in all carrot samples in which *Escherichia coli* was predominant. Contamination with S*taphylococcus aureus* has been linked to carriage in nasal passages of food handlers or by infected workers, (Beuchat, 1995), presence of *Escherichia coli* in the carrots analyzed is indicative of faecal contamination, *Escherichia coli* are part of the normal flora of the human intestines, *Salmonella* spp, and *Shigella* spp, are usually associated with water contamination, contamination with these organisms could arise from washing carrots with contaminated water or handling of carrots by infected workers, (WHO 2002).

Contamination with fungi such as *Fusarium spp, Aspergillus niger, Penicillium spp,* and *Rhizopus spp,* can be attributed to poor storage associated of the carrots, (Scheil 1998).

The disparity observed in the microbial load of carrots obtained from five different vendors may be a direct reflection of the sanitary quality of the water, harvesting, transportation, storage, and processing of the carrots as well as improper handling by individual vendors or processors, (Beuchat, 1996; Ray and Bhunia, 2007).

The high bacteria and fungi counts observed in the carrots in this study are similar to those obtained in other studies in Nigeria (Uzeh et al; 2009; Bukar et al; 2010).

Acetic acid (vinegar) treatment reduces the risk of food borne illness associated with potentially contaminated carrots. Vinegar may serve as a simple and inexpensive disinfectant for carrots in Nigeria, as experimented in this study.

The efficacy of this method used for microbial load reduction is usually dependent on the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of cleaner or sanitizer, pH, and temperature (Parish et al; 2003). Increases in concentration and exposure time were found to be significant in the role of vinegar as a decontaminant for the reduction of microbial population on carrots as observed in this study. The observed proportionate reduction in microbial loads with increase in vinegar concentration

can be attributed to the further reduction in pH resulting from increased vinegar concentration. Most bacteria survive in alkaline pH better than acidic pH. Furthermore, the progressive reduction in microbial loads with increase in exposure time may be due to continuous exposure to this unfavourable pH.

Despite the high microbial counts obtained from some of the carrot samples in this study, it is important to note that these samples did not show any visible signs of contamination. Thus outward appearance may not be a good criterion for judging the microbial quality of fruits and vegetables. All carrots should therefore be adequately washed before consumption either by the consumer or the processor and where possible, decontaminants such as vinegar should be included in the wash water.

5.2 CONCLUSION AND RECOMMENDATIONS

In conclusion, the high bacterial and fungi load in the carrot samples could serve as an indicator for the need to promote awareness about the possible health hazards that could be due to poor handling of these carrots. To limit the introduction of pathogenic bacteria and fungi to carrots through irrigation, the origin and distribution of irrigation water should be known. Where wells are used, such wells should be well-maintained, and all irrigation sources should be monitored routinely for human pathogens (Buck et al; 2003). There is also, the need for regulatory bodies to

ensure that microbiological standards are established and practiced by farmers and marketers for the handling and distribution of carrots.

Carrot processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of contamination. Processors or vendors should also observe strict hygienic measures to ensure that they do not serve as source of chance inoculation of microorganisms and contamination.

Manure used as fertilizer should be treated either by composting or aging to eliminate pathogenic microorganisms and farmers should be educated on the need to allow sufficient amount of time between the final manure application and harvest.

Acetic acid (vinegar) treatment should be used as a decontaminant by either the vendor or consumer so as to reduce the risk of food borne illness associated with carrots.

The establishment of effective GAP (Good Agricultural Practices), and HACCP (Hazard Analysis Critical Control Point) programmes that would cover all aspects of growing, harvesting, packing, transport, processing, distribution, and preparation of carrots is strongly encouraged. Since contamination of raw produce with pathogens can occur at any point in the system, all stages must be considered when devising HACCP programmes, (Tamminya 2010).

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